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THE ANATOMY OF *SIPHONOPHORA ROSARUM*, WALK., THE "GREEN-FLY" PEST OF THE ROSE-TREE.

PART II. THE WINGED VIVIPAROUS STAGE COMPARED WITH THE APTEROUS VIVIPAROUS STAGE.

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(Plates I and II, and two Text-figures.)

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Introduction.

IN Part I (1909) of this paper it was intimated that the author intended to make a comparison between the anatomy of the apterous viviparous form and that of the winged viviparous form. It is clear that these two stages are admirably suited to exhibit the structural modifications which are intimately connected with the habit of flight, for both stages being viviparous parthenogenetic females, no differences are likely to arise due to different modes of reproduction. Investigation shows that the differences are very small; and the points of interest due to one stage possessing the power of flight, consist mainly of the necessary accommodation for the extensive musculature of the wings, the additional nervous supply which the presence of these muscles entails,

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and the presence of certain sense organs not present in the apterous form. These points are summarised at the end of the paper.

I wish again to express my gratitude to Mr A. E. Shipley, F.R.S., and Mr L. Doncaster for help given during the progress of the work, and to Professor F. W. Gamble, F.R.S., and Dr C. Gordon Hewitt for kindly reading and revising the manuscript.

Method.

The insects were fixed in exactly the same way as was stated in Part I, and the usual serial sections obtained.

The winged stage being so much smaller than the apterous stage, the difficulties of actual dissection were materially increased, and this method had finally to be abandoned. In order to obtain figures of the nervous system and alimentary canal, reconstructions had to be made. In the case of the nervous system, the greatest transverse measurement of the nervous system in each of the sections of a complete series was obtained by means of the camera lucida. These distances were then plotted out on squared paper—one half on each side of a middle line. The distance along the middle line between any two of the measurements represented the thickness of the sections at the same magnification at which the measurements were taken. By joining up the points thus obtained, a plan of the nervous system resulted. For the alimentary canal a different method was adopted. Each part of the alimentary tract was considered separately, and the number of sections counted in which the different portions appeared. The figures thus obtained represented the relative lengths of the various divisions; and having determined the transverse dimensions at the magnification at which the reconstruction was to be made, a semi-diagrammatic drawing, to scale, was made on squared paper. Text Figs. 1 and 2 (p. 7) are the average result of several such sets of figures obtained from several complete series of serial sections.

The External Characters.

Buckton's (1875–1882) figure and account of the winged stage of *S. rosarum* seem to contain some small inaccuracies when compared with the actual specimens. These inaccuracies will be dealt with in the general description. His diagnosis is as follows:

> "Green, not bristly. Heads and thoracic lobes black. Abdomen with six or more black transverse bars, decreasing in length and breadth towards the apex.

Four lateral spots on each side. Cornicles green and cylindrical. Tail green. Legs somewhat short, green, with black femoral and tibial points. Wings rounded, with warm blackish veins which are slightly expanded at their outer margins. Cubitus greenish. Stigma grey and imbricated. Third joint of antenna shows a tendency to become ringed. Only the legs and antennae are pilose, and the hairs are non-capitate. Eye red. Rostrum reaches to the second coxae."

The divisions of the body are a little more distinct in this stage than those of the apterous stage. The head is distinctly marked from the thorax, but the line of demarcation between the third thoracic segment and the first abdominal segment is not at all clear. Also the segments of the body have a marked antero-posterior inclination, so that the ventral portion of the last thoracic segment lies vertically beneath the dorsal portions of the first few abdominal segments. As Buckton states, the general colour is green with black markings. The head is deeply pigmented all over; but the first thoracic segment is not so deeply coloured; while the raised dorsal portion, and the shield-like ventral portion of the second thoracic segment, are again deeply pigmented. The third thoracic segment has practically no dark colouration on the dorsal surface, and but little on the ventral surface, between the coxae of the legs. The abdomen has usually seven black stripes or bars across it: the first is broken in the middle, and the next three are almost fused together to form an irregularly shaped black patch on the abdomen. This is a characteristic feature of the insect. The next stripe is considerably shorter than the preceding ones, and not so darkly coloured. The last two stretch from margin to margin of the narrowing apex of the abdomen.

The cornicles are greyish black and not green as Buckton states; and he is also inaccurate in saying that only the legs and antennae are pilose, and the hairs are not capitate, for there are two rows of hairs on the dorsal side of the abdomen (see Plate I, Fig. 1), and these, with many of the hairs on the antennae, are distinctly capitate. It is true that the hairs on the body are not very obvious, and certainly do not give the insect that bristly appearance which the apterous form has.

The legs are not so short in comparison with the body as Buckton seems to have inferred. But the remarkable feature is the extraordinary distance between the first and second pairs (see Plate I, Fig. 2); this is due to the enlargement of the mesothorax to accommodate the wing muscles. The coxa and trochanter of each leg are pigmented in addition to the black "femoral and tibial points."

The mesothorax presents one or two features of interest. The dorsal surface is raised into two conspicuous domes (Plate I, Fig. 1) which are very deeply pigmented and present a shiny appearance in a bright light. On the ventral surface the pigmentation takes the form of a shield upon which are two grooves in the chitin in the form of a cross (see Plate I, Fig. 2). These grooves—more particularly the one in the body axis—indicate the presence of invaginations of the chitin to form strong ridges of chitin (endosternites, or apodemes), which project from the ventral wall of the thorax into the thoracic cavity, and to which the muscles which move the limbs are attached.

The antenna is peculiar in that the third joint has a number of tubercles scattered over its surface; and the sixth joint possesses the same curious ridges which are present in the apterous stage. These features will be dealt with in detail when considering the sense organs. It is probable that it is to these tubercles that Buckton refers when he states that the third joint of the antenna has a tendency to become ringed.

The Wings.

The wings of *S. rosarum* in this stage are, in common with most Aphides, large in comparison with the size of the body. The fore wings are much larger than the hind wings; and when at rest, the wings are folded in the peculiar way which is characteristic of Aphides. During the process of folding, the internal edges of the wings approximate, and at the same time are raised, so that the costal margins become ventral to them, and lie along the insect's back. The result is that the fore wings form a kind of pent-house—of which their internal margins form the ridge—enclosing the hind wings which are similarly folded. The process of folding is performed by several small muscles which are situated at the insertions of the wings. The wings are attached to the more strongly chitinized portions of the thoracic wall by a thin alar membrane which is a continuation of the upper and lower integuments of the wings.

The Wing Muscles.

Packard (1898), in his *Text-Book of Entomology*, gives an account of the various systems of muscles found in different types of insects; and taking his account as representative, the musculature of the wings in this stage of *S. rosarum* is comparatively simple. The wing muscles may be divided primarily into two distinct sets; one set taking up an obliquely dorso-ventral position, and the other stretching in a longitudinal horizontal direction along the dorsal portion of the thorax.

The dorso-ventral muscles consist of four pairs—two pairs on each side of the body. Considering now only the two pairs of muscles of one side, it will be seen (Plate II, Fig. 2) that they occupy the greater part of the half of the thoracic cavity in which they lie, and are arranged with one pair internal to the other; and all of them are markedly oblique—antero-posteriorly and laterally—in their direction, so that in a transverse section through the middle of the thorax, all four belonging to that side will appear (see Plate II, Fig. 2). The outer pair—one, of course, belonging to each of the wings of the side under consideration—may be said to have their origin in the chitin of the floor of the thoracic cavity, and are inserted into the inflexed portion of the thoracic wall which occurs at the insertion of the wings (Plate II, Fig. 3). The inner pair have their origin in the floor of the thorax, a little nearer to the mid-ventral line than the previously mentioned pair, and are inserted into the dorsal wall of the thorax, where the dorsal surface merges into the lateral one. The longitudinal horizontal muscles all lie in the dorsal portion of the thoracic cavity, and consist in all of eight separate muscles (see Plate II, Fig. 2), arranged in four pairs—one pair above another. Taking them in order from above downwards, the first pair lie closely applied to the under side of the dorsal wall of the thorax; each muscle lying a little to the right or left of the median dorsal line. The remaining three pairs are grouped together, are more deeply seated than the preceding pair, and lie much closer together. All these muscles are attached at their anterior ends to the front margin of the prothorax, and posteriorly to the hind wall of the metathorax.

The action of the muscles. In order to make the action of the wing muscles quite clear, it will be advisable to consider first the action of each muscle individually, and then consider their action as a whole. Commencing with the outer pair of dorso-ventral muscles on each side, it will be obvious from Plate II, Figs. 2 and 3, that the contraction of these muscles will drag down the inflexed piece of chitin into which the muscle is inserted, and consequently exert a pull upon the lower portion of the alar membrane which is attached to it. Provided that the dorsal portion of the body wall remains rigid, the effect of this pull will be to depress the wing. These muscles may therefore be called the "Depressor Muscles." Similarly, provided that the depressor muscles remain in a passive, uncontracted state, the contraction of the inner dorso-ventral muscles will have the effect of elevating the wing, because the pulling down of the "crowning arch" of the thorax will cause a pull to be exerted upon the upper portion of the alar membrane, and the junction of the lower portion of the alar membrane acting as the fulcrum, the wings will be elevated. These muscles may therefore be called the "Elevator Muscles." Coming now to the longitudinal muscles, it is probable that they perform, or assist in the performance of, some depressor function. The dorsal portion of the thorax being so strongly chitinized, will not allow of much movement of a telescopic character, so that the contraction of the longitudinal muscles—which, it will be remembered, are attached to the anterior and posterior faces of this dorsal arch—will have the effect of decreasing the distance between these faces; and as telescopic contraction is not possible, the only alternative is that the transverse measurements of this portion of the thorax will be increased. This can be illustrated in a concrete manner with the half of an india-rubber ball. If the ball be compressed along one diameter—which will represent the longitudinal axis of the thorax—the length of the diameter at right angles to the line of compression—representing the transverse axis of the thorax—will be increased. The effect of the increase in length of the transverse axis of the thorax will be to carry the point of attachment of the dorsal portion of the alar membrane beyond the point of attachment of the ventral portion. The total effect being that the wings will be depressed. The insect therefore possesses two sets of muscles for depressing the wings, and one set for elevating them. The actual sequence of contractions is probably as follows: First the small muscles attached to the insertions of the wings will cause the wings to leave their inverted, folded-up position over the back, and arrange themselves at right angles to the longitudinal axis of the body; the whole of the longitudinal muscles and the depressor muscles will then contract, imparting to the wings a powerful down-stroke. These muscles then relaxing, the elevator muscles will contract and raise the wings to their former position, and the cycle will begin again.

The Alimentary Canal.

The alimentary canal of the winged stage of *S. rosarum* (Text Figs. 1 and 2) exhibits some points of interest. The thorax of the insect is almost completely filled up with the wing muscles, and as a natural consequence, the alimentary canal in this part of the body is crushed together in a dorso-ventral direction, so that the various coils lie closely beneath one another. The coils also differ in arrangement from those present in the apterous form; but there is a general similarity between the form of the alimentary canal in the two stages.

The course of the alimentary canal. Commencing at the mouth, the oesophagus passes in an upward direction towards the roof of the head, and reaching a position a little above the median vertical point, it curves abruptly backwards as is the case in the apterous stage, and passing over the transverse bar which supports the chitinous framework of the head, it continues in the median line into the region of the thorax. Here it gives place to the crop, which, however, does not lie obliquely in the body, but continues parallel to the median line. The crop is followed in the usual way by the coiled intestine, and it is in this portion of the alimentary canal that differences arise. The coiled intestine after leaving the crop makes a curve towards the left side, and at the same time dips towards the ventral surface of the body cavity. Instead of curving completely round and proceeding in an anterior direction as is the case in the apterous stage, the coiled

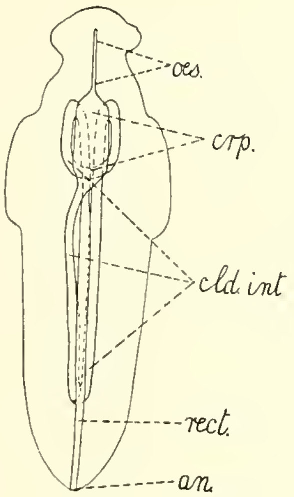


Fig. 1.

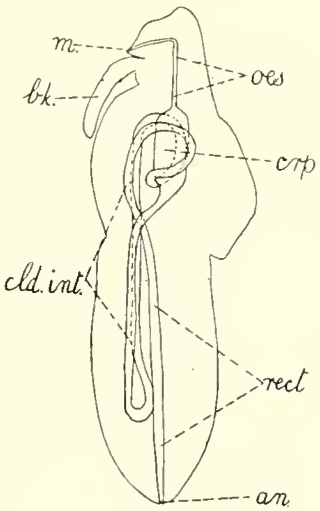


Fig. 2.

Fig. 1. The alimentary canal. Dorsal view. Semi-diagrammatic. (× 40.) *an.* = anus. *cld. int.* = coiled intestine. *crp.* = crop. *oes.* = oesophagus. *rect.* = rectum.

Fig. 2. A lateral view of the alimentary canal. Semi-diagrammatic. (× 40.) *an.* = anus. *bk.* = beak or proboscis. *cld. int.* = coiled intestine. *crp.* = crop. *m.* = mouth. *oes.* = oesophagus. *rect.* = rectum.

intestine continues in its backward course until it approaches the posterior end of the body. It now makes a curve upwards and forwards and proceeds in an anterior direction into the region of the crop, and at the same time returning to the ventral surface, where it lies on the right side of the first portion. Having reached the region of the beginning of the crop, the intestine makes another upward curve and then proceeds backwards at the right side of the crop for its whole length. It then makes an abrupt curve downwards, at the same time encircling the crop, and appears eventually at the same level on the left-hand side. It now takes an anterior course and passes towards the region where the oesophagus joins the crop, but not quite so far as was the case on the right-hand side. Here it makes its final curve downwards towards the ventral surface, and passing through the loop formed by the first two coils, it takes a direct course to the anus.

It is clear at once that there is a similarity between the alimentary canal in this stage and the apterous stage, but one which is more superficial than real. The main differences consist of the absence of the first coil present in the apterous individuals, and the curious complication of the second coil. It is difficult to see, on the one hand, why the alimentary canal should have these curious coils in this stage; and on the other hand, why the elimination of the habit of flight should be coincident with such a rearrangement of the coils. It is quite conceivable, of course, that the form of the alimentary canal has no connection with the difference of habit, and may be due to other causes which are at present unknown.

The *mouth* and *mouth organs* are almost exactly the same as in the apterous stage, the differences being very small and probably having no effect upon the functions of the various parts.

The *oesophagus* offers no points for discussion beyond the fact that the lumen is perhaps relatively a little wider than it is in the apterous stage.

The *crop* is perhaps a little longer in proportion to its width than it is in the apterous stage. The usual oesophageal valve, where the oesophagus enters the crop, is present.

The *coiled intestine* varies very much in different individuals in the size of cross section and of the lumen. In many instances it resembles the coiled intestine of the apterous stage, but in some cases the lumen is reduced, in cross-section, to a star-shaped space in the centre of a rounded mass of granular material. These differences may be only post-mortem developments, but they are confusing.

The *rectum* is also very variable in different individuals. When no contraction has taken place, the lumen is rounded in contour, and bounded by the usual thin muscular wall. Where contraction has occurred, the rectum becomes stellate in cross-section. A sphincter muscle, formed by the thickening of the muscular wall, closes the anus as in the apterous form.

The *salivary glands* are exactly similar to those present in the apterous stage, and the ducts take the same curious course round the nervous system, uniting in the mid-ventral line, and then continuing forward underneath the nervous system to the mouth.

The Nervous System.

The comparison of the nervous systems of the two stages is rather disappointing, because it would be expected that such a vital difference in habit would involve some marked changes in the nervous system. The slight differences which are present will be dealt with in the description of the various parts. In general appearance, the central nervous system (Plate II, Fig. 4) is very similar to that of the apterous individuals. The same ganglia, viz., two cerebral ganglia, each with its adjacent optic lobe or ganglion, two sub-oesophageal ganglia, three pairs of thoracic ganglia, and a single median abdominal ganglion, are present. The ganglia are connected up in exactly the same way as is the case in the apterous stage, but the whole system appears to be much more contracted and concentrated.

The *cerebral ganglia* and their optic lobes or ganglia, are larger in proportion to the rest of the nervous system. This is, of course, the natural outcome of the different habit of the insect in this stage, for it is reasonable to suppose that the habit of flight would be accompanied by a greater demand upon all the organs of sense, and more especially upon the eyes and any organs which are adapted to appreciate waves in the air. These organs, placed in close relation with the head, are innervated by the cerebral and optic ganglia enclosed in the head. The cerebral ganglia are joined to the sub-oesophageal ganglia by a pair of circum-oesophageal connectives, which arise, as in the apterous form, one from each of the lower and tapering portions of the cerebral ganglia; and passing one on each side of the pharynx, become continuous with the sub-oesophageal ganglia.

The *sub-oesophageal ganglia* need no comment other than that my investigations have failed to reveal any nerve given off from these ganglia to the muscles of the mouth-organs, as is the case in the apterous form. This, however, does not disprove that such a nerve is present, and moreover the nerve in the apterous form is very fine, and not easily traced in transverse sections. It is probable that its minuteness has prevented its discovery.

The *thoracic ganglia.* At first sight it would seem as if there was very little difference in this part of the nervous system. The thoracic ganglia are connected by a similar elongated portion with the suboesophageal ganglia, and a pair of ganglia are present for each of the three segments of the thorax. The remarkable feature about the ganglia is the concentration which has undoubtedly taken place from the typical nervous system, so that the whole of the thoracic ganglia and the median abdominal ganglion lie almost entirely in the prothoracic segment, extending but a short distance into the mesothorax. The consequence of this crowding is that the ganglia lie partially one above another; so that the posterior portion of the first thoracic ganglia lies underneath the anterior portion of the second thoracic ganglia. The second and third thoracic ganglia lie in like relation.

If the view be taken, and it is the most probable one, that the apterous stage is a degeneration from the winged stage, it will appear that the elimination of the habit of flight has merely caused a slight step back towards the type-form of nervous system, in that the ganglia in the apterous stage are not so crowded together, and that a gradual relative elongation of the system has taken place. The crowding of the ganglia in the winged stage is therefore probably due to some evolutionary influence, and may not have any connection with the presence of organs of flight. It must, however, be borne in mind that from the point of view of the nervous system, it is just as feasible to suppose that the winged form is an advance on the apterous form, and that the introduction of the habit of flight has made greater demands on the thoracic ganglia, and that the increase in size necessitated by this increased demand has resulted in the crowding of the ganglia together.

The nerves from the thoracic ganglia are distributed in a different manner from that described in the apterous form. This is due to the extraordinary development of the mesothorax to accommodate the wing muscles. The effect of this enlargement is that the first and second pairs of legs are much further apart than are the second and third pairs. Consequently, while the nerve from each of the first thoracic ganglia leaves its ganglion almost at right angles to the axis of the body, the nerves from the second and third thoracic ganglia are given off from their posterior margins, and follow a course almost parallel to the body axis, extending for some distance along the thorax before reaching their respective destinations. The nerve from each of the second thoracic ganglia travels for some distance in the nuclear sheath which surrounds the ganglia themselves before finally entering the thoracic cavity.

Besides supplying nerves to the legs, the thoracic ganglia innervate the wing muscles, but as far as my investigations show, these muscles receive the whole of their nerve supply from the second thoracic ganglia. The nerves which proceed from these ganglia give off a branch which passes towards and is lost amongst the wing muscles, but I have been unable to discover a similar nerve given off from the nerves from the third thoracic ganglia.

The *median abdominal ganglion* gives off one large nerve which, as in the apterous stage, runs along the mid-ventral line nearly to the end of the abdomen where it expands into a small ganglion.

Sense Organs.

Consideration of the nervous system naturally leads to the sense organs. The only definite sense organs which the winged stage possesses are the eyes and the antennae.

The eyes in this stage—as would be expected—are larger in proportion to the size of the head than in the apterous stage. From Plate I, Fig. 3, it will be seen that each eye occupies a considerable portion of the side of the head, and also that the supplementary eye is much more distinct and consists of three small facets. The number of facets in the eye is larger, there being on an average about eighty facets in the winged stage and sixty in the apterous stage. This enlargement accounts for the increased size of the optic ganglia.

The antennae present many features of interest. As in the case of the apterous form, the last or sixth joint has a number of curious ridges and markings on it; but where the expanded portion gives place to the tapering portion, there are, in the winged stage, several curious tubercles or excrescences, similar to those which will be described later as found upon the third joint of the antennae. At the junction of the fifth with the sixth joint, there is another of these curious tubercles. These points are illustrated in Plate I, Figs. 5 and 6. Whatever kind of sense organ the peculiar ridges on the last joint may represent, it is clear that these new structures must be looked upon as satisfying some need which has been brought about by the presence of the habit of flight. As has been mentioned before, the third joint (Plate I, Fig. 4) has its entire surface covered with a number of small, clear, dome-shaped tubercles, which vary somewhat in size, and give a most remarkable appearance to the joint. Each tubercle consists of a blister-like excrescence on the chitin of the joint, and the epidermal lining is continued into the cavity thus formed. The sides of the tubercle (see Plate I, Fig. 5) are pigmented like the surrounding surface of the joint, but the top is quite clear, and has the appearance of a membranous covering to a short cylinder of chitin. In nature, the top is almost hemispherical in shape, but in the section represented in Plate II, Fig. 5, considerable contraction has taken place so that the thin portion has become flattened and distorted. The cavity of the tubercle is filled up with a group of cells which seem to be modified epidermal cells which have taken on a sensory function. These cells are expanded at their base and taper off as they near the thin covering of the tubercle.

It is of course almost impossible to say what kind of sensory organ these tubercles represent, but one thing is certain, that, as they are not present in the degenerate apterous form, they must meet some need which is experienced during the period when the insect possesses the power of flight. It does not follow, however, that these organs are directly connected with the actual process of flying; because flight, in giving to the insect the power of migrating from one host-plant to another, may create a need for sense organs to enable it to recognise the correct host-plant suited to its wants. Possibly these organs satisfy that need; without some such sensory apparatus it is difficult to see how these winged individuals can find the correct plant upon which to carry on their life cycle, for it is not conceivable that such an important matter would be left entirely to chance.

The Tracheal System.

Owing to its having proved impracticable to obtain good preparations of the tracheal system of the winged stage, no detailed comparison of the two stages can be made. From the very imperfect preparations that it was possible to make, it would seem that in the abdomen, the system is a little more complicated than in the apterous stage, but chiefly in the larger number of fine branches given off from the larger tracheae. The thickness of the thoracic wall and the presence of dark pigment made it impossible to obtain any idea of the arrangement of the tracheae in the thorax, but branches could be distinguished passing to the head and antennae much in the same way as in the apterous form. The stigmata, however, are clearly visible on the external surface and consist, as in the apterous stage, of two pairs of thoracic stigmata, and seven pairs of abdominal stigmata. The thoracic stigmata are situated, one pair in the prothorax and the other pair in the metathorax, and the abdominal ones occupy the first seven abdominal segments, one pair in each segment. An examination of transverse sections shows that, in the abdomen at any rate, each stigma opens into a single main trachea, which in this stage is more dilated than in the apterous form, and constitutes an air chamber, from which two main branches take their origin, the one passing dorsally and the other ventrally, and presumably joining up with similar branches from other stigmata to make up a dorsal and ventral tracheal system as is present in the apterous form.

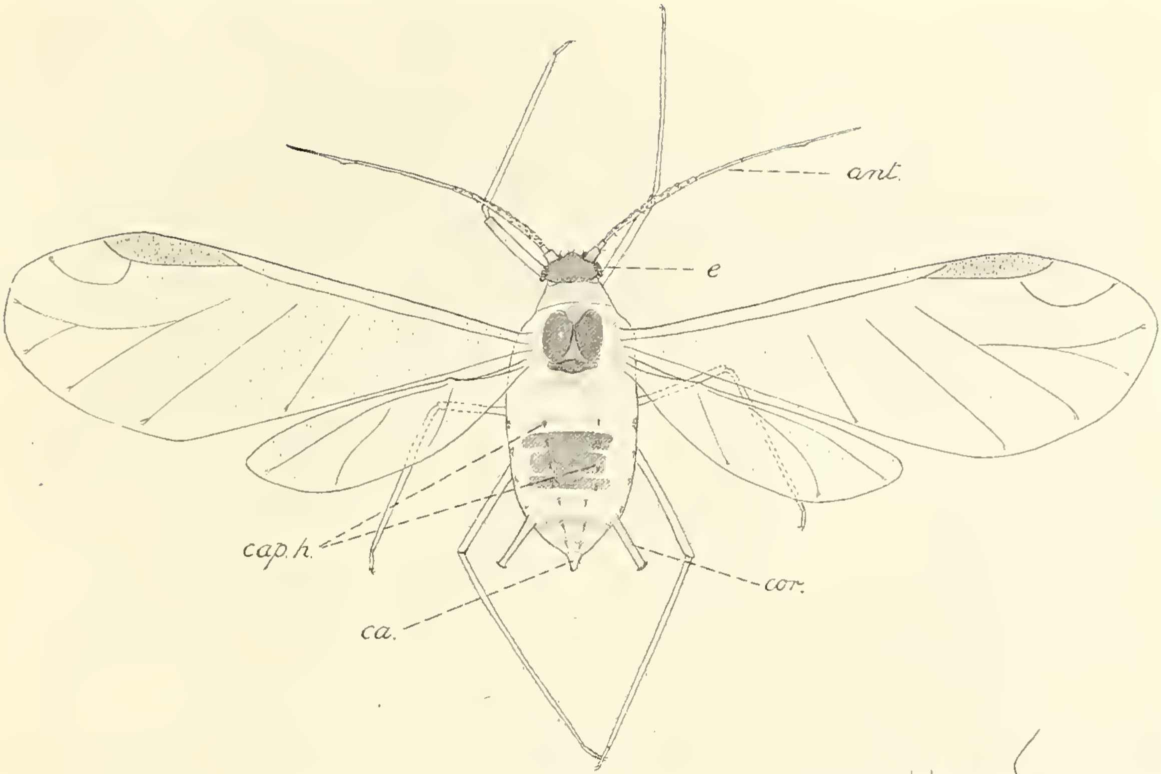
Summary

The Apterous Parthenogenetic Viviparous Female.	The Winged Parthenogenetic Viviparous Female.
External Characters.	*External Characters.*
Segmentation indistinct.	Segmentation more distinct, mesothorax much enlarged.
Hairs very numerous; six rows on the thorax and abdomen; capitate.	Hairs less numerous; only two rows on the abdomen; capitate.
No black pigment.	Many patches of black pigment on the body.
	Wide separation of the first and second pairs of legs.
	The Wing Muscles.
	A single elevator and depressor muscle for each wing, and a set of eight longitudinal muscles which assist in depressing the wings.
The Alimentary Canal.	*The Alimentary Canal.*
The coiled intestine describes two backward coils and one forward coil. The first backward coil occurs immediately after leaving the crop; then, after forming the forward coil, which occurs at the posterior end of the body, the intestine again reaches the region of the crop where the second backward coil occurs just before the commencement of the rectum. All the coils are in a horizontal direction.	The first backward coil is completely absent, and where the second backward coil should occur, there is a complicated coil consisting of first a vertical coil on the right hand side of the crop then a horizontal coil passing beneath the crop followed by another vertical coil just before the coiled intestine gives place to the rectum.

Summary (cont.).

The Apterous Parthenogenetic Viviparous Female.	The Winged Parthenogenetic Viviparous Female.
Nervous System.	*Nervous System.*
Great concentration of the ganglia, especially of the thoracic ganglia.	Concentration more marked than in the apterous form and also a relative increase in the size of the ganglia. The wing muscles are apparently only supplied by the nerves from the second thoracic ganglia.
Sense Organs.	*Sense Organs.*
The sixth joint of the antenna is covered with curious markings.	Ridges are present on the sixth joint of the antenna and the third joint is covered with a number of tubercles. A few tubercles are also present on the fifth and sixth joints.
Compound eyes (about 60 facets) with a small supplementary eye.	Eyes larger than in the apterous form (about 80 facets) and the supplementary eye is much more prominent.
Tracheal System.	*Tracheal System.*
A complicated dorsal and ventral system present formed by the division of the main trachea from each stigma into dorsal and ventral branches which join up to form the respective systems.	No details of the systems obtainable, but the main trachea from each stigma divides in exactly the same way as in the apterous form.

The foregoing comparison of the anatomy of the apterous viviparous and the winged viviparous stages of *S. rosarum* although not revealing any very great differences, may be regarded as supporting the view that the apterous stage is a degeneration from the winged stage. The fact that so much similarity exists between the two forms is perhaps more easily explained by supposing that the habit of flight had become eliminated, possibly through disuse, resulting in the consequent atrophy and disappearance of the organs directly concerned with that function; for if the other view be considered, it would be reasonable to suppose that more specially marked differences in an upward direction would be the result of such a vital change in the habit of the creature. There is one point of difficulty, however, and that is to account for the difference in form of the alimentary canal, but, as has been already suggested, this may be due to other, quite different, and at present unknown, causes.



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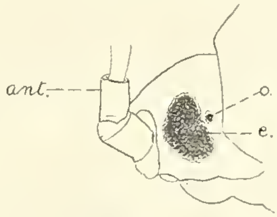

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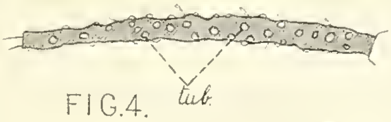
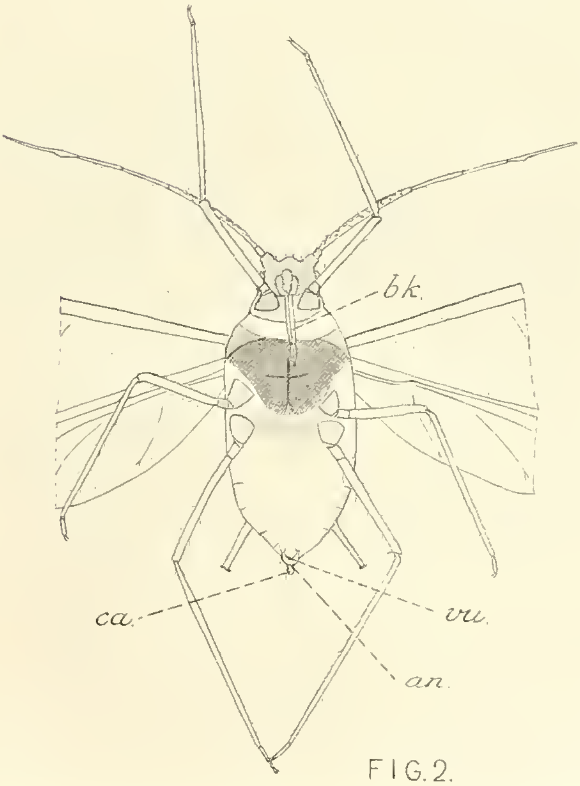


FIG. 4.



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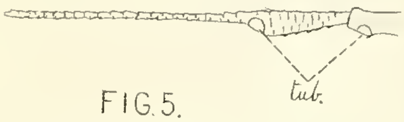

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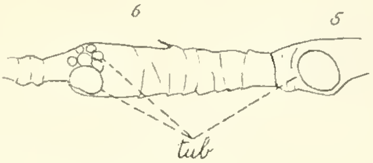

FIG. 6.

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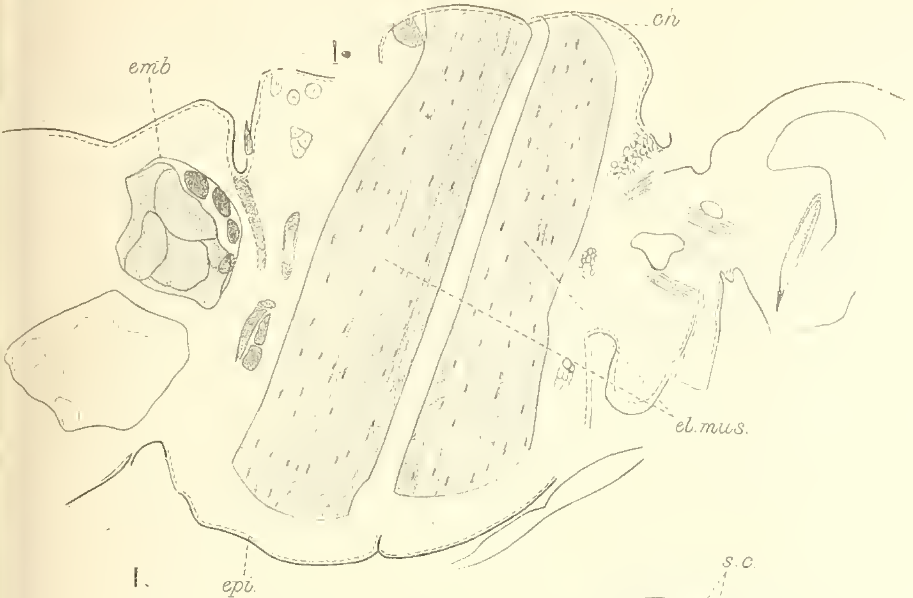

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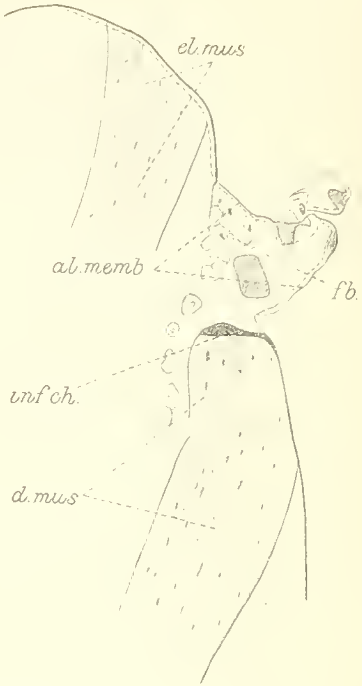

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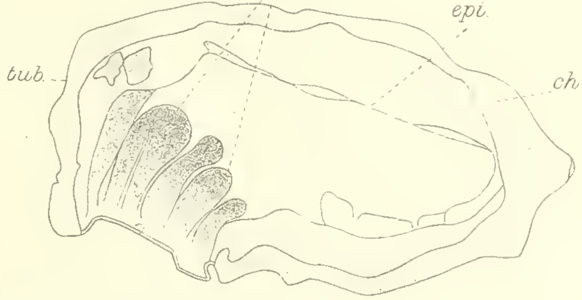

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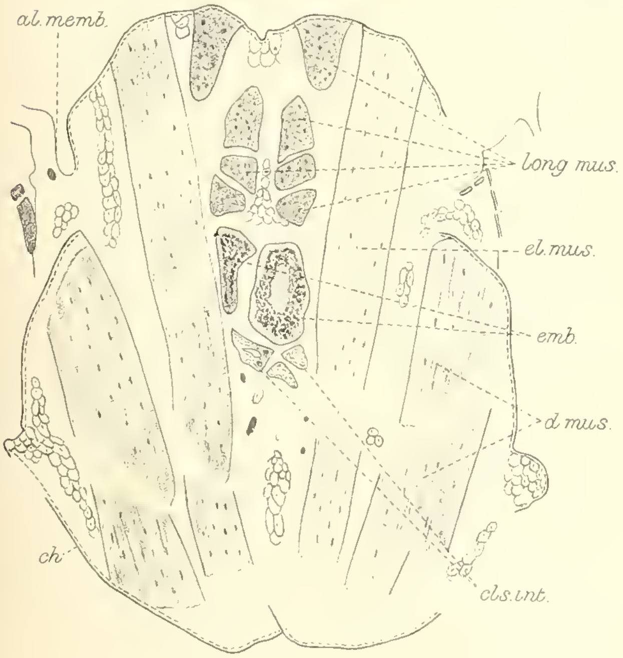

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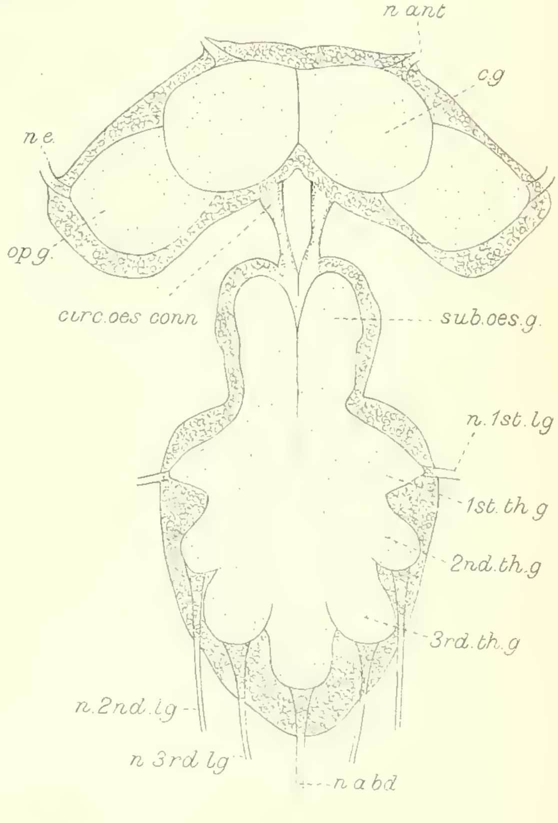

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EXPLANATION OF PLATES I AND II.

All drawings were made with the Camera Lucida unless otherwise stated.

PLATE I.

Fig. 1. The winged viviparous stage of *Siphonophora rosarum*. Dorsal view. (× 21.)

Fig. 2. The winged viviparous stage of *S. rosarum*. Ventral view. (× 21.)

Fig. 3. The eye, seen from the side. (× 72.)

Fig. 4. The third joint of the antenna, to show the curious tubercles scattered over its surface. (× 72.)

Fig. 5. The sixth joint of the antenna. (× 100.)

Fig. 6. Portions of the fifth and sixth joints of the antenna seen from below. (× 233.)

PLATE II.

Fig. 1. Longitudinal section through the thorax to show the elevator muscles of the wings. (× 108.)

Fig. 2. Transverse section through the thorax to show the arrangement of the wing muscles in the thoracic cavity. (× 108.)

Fig. 3. The insertions of the elevator and depressor muscles of one side. (× 152.)

Fig. 4. The central nervous system as seen from above. (Semi-diagrammatic.) (× 178.)

Fig. 5. Transverse section across the antenna including one tubercle. (× 1050.)

REFERENCE LETTERING.

al memb.	Alar membrane.
an.	Anus.
ant.	Antenna.
bk.	Beak or proboscis.
ca.	Cauda.
cap. h.	Capitate hairs.
c. g.	Cerebral ganglion.
ch.	Chitin.
circ. oes. conn.	Circum-oesophageal connectives.
cls. int.	Coils of the intestine.
cl. mus.	Depressor muscle of the wings.
cor.	Cornicle.
e.	Eye.
el. mus.	Elevator muscle of the wings.
emb.	Embryo.
epi.	Epidermis.
fb.	Fat-body.
inf. ch.	Inflexed piece of chitin into which the depressor muscle is inserted.
long. mus.	Longitudinal muscles which assist in the depressing of the wings.
n. abd.	Abdominal nerve.
n. ant.	Nerve to the antenna.
n. e.	Nerve to the eye.
n. lg. 1st, 2nd, 3rd	Nerve to the 1st, 2nd and 3rd legs respectively.
o.	Supplementary eye.
op. g.	Optic ganglion or lobe.
s. c.	Sensory cells.
sub. oes. g.	Sub-oesophageal ganglion.
th. g. 1st, 2nd, 3rd	1st, 2nd and 3rd thoracic ganglia.
tub.	Tubercle on the antenna.
vu.	Vulva.

THE DIVISION AND POST-FISSION MOVEMENTS OF BACILLI WHEN GROWN ON SOLID MEDIA.

By G. S. GRAHAM-SMITH, M.D.

(*University Lecturer in Hygiene, Cambridge*).

(Plates III—VIII and 14 Text-figures.)

THE behaviour of living bacteria during artificial cultivation in fluid and semi-solid media has often been observed and described, but very few investigators appear to have studied the changes which take place when bacteria are grown either on the surface or in the depth of solid media. The investigations which are described in this paper were undertaken with the purpose of ascertaining how various bacilli behaved during the early stages of growth on the surface and in the depth of agar, and how far the various types of division and post-fission movement influenced the characters of the colonies subsequently formed.

Hill (1901) devised a method for watching the growth of bacteria on agar and carefully described the mode of growth of *B. diphtheriae* and of *B. typhosus*. His "technique consists briefly in substituting for the ordinary 'hanging drop' of liquid or jelly a cube of solidified agar, on the surface of which the bacteria are distributed. The inoculated surface of this cube is applied to the under surface of a cover-slip, and for convenience is known then as the 'hanging block.' Oxygen probably reaches the bacteria by diffusion through the block or the seal. Certainly aerobic bacteria like *B. diphtheriae, B. typhosus*, etc., grow readily in such preparations."

Hill (1902, p. 204) gives the following directions for preparing hanging block preparations. "Pour melted agar into a Petri dish to the depth of about one-eight to one-quarter inch. Cool this agar and cut from it a block about one-quarter inch to one-third inch square and of the thickness of the agar layer in the dish. This block has a smooth under and upper surface. Place it, under side down, on a slide and protect it from the dust. Prepare an emulsion in sterile water of the organism to be examined, if it has been grown on a solid medium, or use a broth culture; spread the emulsion or broth upon the upper surface of the block as if making an ordinary cover-slip preparation. Place the slide and block in a 37° C. incubator for five to ten minutes to dry slightly. Then lay a clean sterile cover-slip on the inoculated surface of the block in close contact with it, usually avoiding air bubbles. Remove the slide from the lower surface of the block and invert the cover-slip so that the agar block is uppermost. With a platinum loop run a drop or two of melted agar along each side of the agar block, to fill the angles between the sides of the block and the cover-slip. This seal hardens at once, preventing the slipping of the block. Place the preparation in the incubator again for five to ten minutes to dry the agar seal. Invert this preparation over a moist chamber and seal the cover-slip in place with white wax or paraffin. The preparation may then be examined at leisure." In most of Hill's experiments a warm stage was made use of.

The results obtained by Hill (1901) and by Hill and Rickards (1903), who appear to be the only workers who have investigated the growth of bacteria under such conditions, will be referred to later.

METHODS.

1. *Growth on the surface of agar.*

In order to investigate the mode of growth on the surface of agar the following very simple method was employed. A small quantity of melted agar was poured on to the surface of a sterile glass slide and immediately spread with a warm sterile platinum needle, so as to cover at least one square inch of surface. As soon as the agar had solidified a small drop of a very dilute emulsion of the organism to be investigated was placed on the surface of the agar, and a square three-quarter inch cover-glass was then gently lowered over the drop, avoiding as far as possible the formation of bubbles. The best plan of accomplishing this is to place one edge of the cover-glass near the edge of the agar and gently lower the opposite edge with a platinum needle. Next the agar projecting beyond the cover-glass was cut away on all sides[1], and the edges of the cover-glass sealed to the slide all round by means of a small quantity of melted vaseline. The specimen was then transferred to the stage of a microscope placed within a Nuttall's microscope thermostat, kept at 37° C. Such a preparation can be continuously studied for many hours or days.

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[1] It was found that if the cover-glass was only sealed down by means of vaseline on to the surrounding agar (without removing the excess of agar) the preparation gradually contracted and the focus of the microscope had to be constantly altered.

This method is applicable to motile as well as non-motile organisms. The bacteria cannot lie otherwise than horizontally and in the same plane. They are all necessarily in optical contact with the cover-glass and are free to grow (horizontally) without the restrictions imposed by surrounding them with jelly or the freedom to drift allowed if liquid is used.

2. *Growth in the depth.*

In order to study growth in the substance of the agar a small quantity of bacterial emulsion was mixed with melted agar at 40° C. and the latter poured on a slide. When the agar was just beginning to set a cover-glass was placed on it and the specimen finished as in the case of surface preparations.

3. *The bacterial emulsion.*

The emulsion of the bacterium to be investigated was invariably made from a 12 to 18 hour sloped agar culture. In making the emulsion sterile tap water was generally used. Even in the young cultures which were made use of a certain proportion of the organisms were found to be dead, or at any rate incapable of growth under these conditions. This was more frequently the case if a portion of the growth from the surface of the agar culture was used for making the emulsion. The proportion of dead bacteria was much smaller if the growth occurring in the water at the bottom of the tube was used.

4. *The choice of an organism for observation.*

The observations were usually made with a Zeiss 4 mm. objective and an 8 compensating ocular, artificial light being used for illumination, with sub-stage lowered and a narrow diaphragm.

For satisfactory observations a part of the preparation should be sought for where the bacteria are so thinly scattered that only a few are within the field at the same time (see Plate VI, Fig. 13). A well isolated organism, which looks capable of growth and which is lying in a clean field, should be chosen. After a little experience it is generally easy to decide whether a given organism is going to divide or not. An organism which is likely to divide is usually larger and better defined and more opaque than one which is apparently dead. Moreover in some species the living healthy bacterium, if carefully watched, shows slight changes in outline at intervals, suggesting some plasticity in the bacterial walls.

5. *Method of recording the observations.*

After the preparation had been placed on the stage of the microscope and a suitable organism focussed, the time was recorded and the organism drawn and labelled 1. The specimen was then carefully watched, and any changes noted. Usually a long period of time, up to an hour or more, elapses before any change occurs. In most species the first important change to be seen is an increase in length, accompanied by a slight bending. Later a segmentation interval appears in the middle of the organism, dividing it into two more or less equal portions. These two parts are labelled 11 and 12 respectively in the drawings. After this division, or fission, the behaviour of the organism differs according to the group to which it belongs, but in all cases various movements take place to which the term " post-fission movements " was applied by Hill. The two new organisms (11 and 12) grow and again divide, and ultimately numerous organisms are produced and form a colony. For the purposes of identification the organisms arising from the division of 11 were labelled 111 and 112, and those from 12 were labelled 121 and 122 and so on[1].

The time at which each division or other well marked change occurred was also noted.

6. *Photographic records.*

Photographic records were also obtained, showing the mode of multiplication of a typical member of each group. When a suitable bacterium had been found the microscope was removed from the thermostat and clamped in a horizontal position on a Zeiss camera. Illumination was obtained by means of limelight or a mercury vapour lamp, and the organism focussed on a clear glass screen. The magnification varied between 100 and 500 diameters in the different series, as it was found impossible to obtain satisfactory results with the higher magnifications with some species when growing on the surface. Growths in the depth were necessarily only slightly magnified as the organisms do not remain in the same plane. The photographs reproduced in Plates III, IV, V are shown at the original magnification, but some of those on Plates VI and VII have been somewhat enlarged. In all cases the prints were obtained from untouched negatives. Very fast plates were generally made use of in order to reduce the period of exposure as much as possible. If a long exposure is given a certain amount of movement invariably occurs and spoils the negative. The exposures usually varied between five and ten seconds, though sometimes it was necessary to give up to 30, 60 or even 90 seconds.

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[1] The genealogical relationships of the new cells are indicated by Hill's (1901, p. 84) modification of Rickard's (1901) system for culture record. The modification consists merely in omitting the decimal point and all figures to the left of the point.

As soon as the exposure had been made the microscope was taken back to the thermostat and the preparation watched until sufficient development had occurred to render another photograph desirable. The greatest care had to be exercised in moving and clamping the microscope as the slightest jar was apt to completely spoil the specimen, and many were ruined by such accidents.

Owing to the frequent changes in temperature caused by removing the microscope from the thermostat to the camera development was slow, and in order to obtain a satisfactory series the preparation had to be started very early and watched for several hours.

I wish here to acknowledge my indebtedness to my laboratory assistant, Mr J. Charles, for the great help he rendered in the solution of some of the difficulties met with in obtaining these photographic records, and for the care and attention he bestowed on the negatives.

General observations.

In order to satisfactorily follow the various changes which occur in the formation of a small colony from an isolated bacillus several hours have to be devoted to watching a single preparation. Moreover owing to various causes such as interruptions, accidents and unsuccessful preparations it was almost invariably necessary to spend several days watching a number of preparations of a single species. As only occasional days could be devoted to this work the observations have extended over a period of more than two years. During this time the mode of growth of a considerable number of species of bacilli and vibrios has been investigated.

As has already been stated growth in the long axis of the original organism first occurs and then division, followed by post-fission movements. By repeated divisions and movements colonies are produced. In every species hitherto investigated a decided tendency towards the adoption of a parallel arrangement by the newly formed rods has been noted. This parallel arrangement, which may be of short duration or which may persist indefinitely, is brought about by the post-fission movements. Four different well marked varieties of post-fission movement have been observed, and according to their post-fission movements the organisms investigated have been divided into four groups.

I. The "loop forming" group.
II. The "folding" group.
III. The "snapping" group.
IV. The "slipping" group.

The differences in post-fission movement appear to be explicable on the hypothesis that the variations are mainly due to differences in the strength and behaviour of the capsule.

Up to the present however it has been found impossible to make direct observations on the behaviour of the capsule during division in the living state, either with the ultramicroscope or by any other means. That a capsule or membrane exists may be clearly demonstrated for even in crowded preparations individuals lying side by side are rarely or ever in visible contact, an interval always remaining between them. Very little information has been gained by the study of stained specimens.

It was early clearly recognised that the behaviour of an organism growing in a confined space between agar and glass might be totally different from its behaviour when growing freely on the surface of an agar plate. Parallel observations were therefore frequently made by simultaneously watching a specimen prepared by the method described and the growth occurring on the surface of an agar plate made from the same emulsion. While the former was under almost constant observation the latter was taken out of the incubator at frequent intervals and examined. The growth taking place in the two cultures could thus be easily compared. These experiments showed that bacilli growing under ordinary conditions on the surface of agar plates behave for many hours in exactly the same manner as those growing between agar and glass.

Deep colonies.

In each group the deep colonies differ to a greater or less extent from the superficial colonies, and the deep colonies produced by the organisms belonging to the four groups differ from each other in their appearance and mode of production as greatly as do the superficial colonies. Nevertheless the mode of division, the method of post-fission movement and the function of the capsule remain the same in each group whether growth is occurring in the depth or on the surface, and the differences in the deep colonies can be attributed in each case to the same variations in the strength and behaviour of the capsule, which influence the superficial growths.

Detailed account of observations.

Group I. The "loop forming" group.

The only organism belonging to this group which was studied was *B. anthracis.*

During the early stages of growth the original organism increases in length and divides, producing a chain of closely connected rods, which develops a distinct curve near its centre. This primary curve soon develops into a loop, and secondary loops are rapidly produced at the opposite side of the chain. To accommodate the rapidly increasing length of the chain further loops are produced, and the pressure they exert results in the compression of the central portions of the primary, secondary and later loops to such a degree that for long distances their walls are in apposition. Thus long wavy strands of parallel chains of bacilli are produced. Ultimately therefore the colony is composed of a central portion consisting of numerous wavy strands of parallel chains of bacilli running in various directions, and of a peripheral portion formed by the numerous loops in which the strands terminate. The terminal portions of the original thread can generally also be recognised as delicate wavy processes projecting from the colony, and often showing systems of loops like those seen in the earlier stages of growth of the primary colony.

Diagram 1 is reproduced from a series of drawings made during the development of a superficial colony from a single anthrax bacillus. Over each figure the time in minutes from the commencement of the observation is given.

In Fig. 1 the single bacillus is seen. During the first 100 minutes slow growth occurred and the bacillus divided into two bacilli of equal length (Fig. 2). Subsequently growth was much more rapid. After

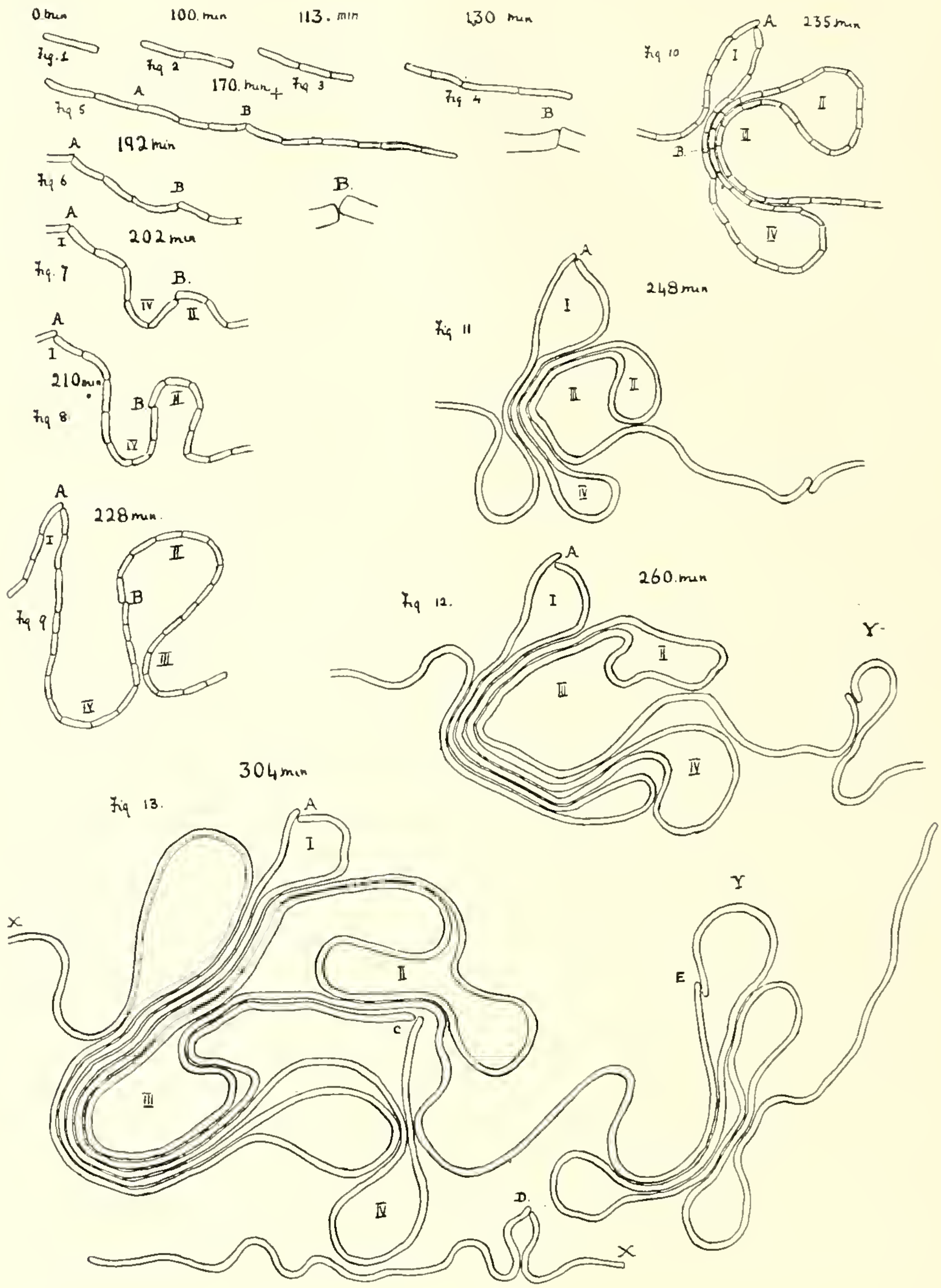

Diagram 1. Illustrating the development of *B. anthracis* on the surface of agar.

130 minutes a chain of four bacilli had been produced (Fig. 4), and after 170 minutes a chain of eleven bacilli (Fig. 5). By this time the chain had developed a decided bend at B, and a less marked one at A. Owing to the fact that the adjacent bacilli slipped slightly out of the line of the chain at these points their positions could be easily recognised during subsequent growth. It is of interest to note that the linear distance separating the points remained approximately the same, as long as the points continued to be recognisable (Figs. 5 to 10). The next four figures (6, 7, 8 and 9) illustrate the formation of a primary (IV) and two secondary (I and II) loops. Fig. 10 shows further development of these loops and the addition of another (III), and also illustrates how the pressure of the developing loops causes the formation of parallel chains of bacilli. The development of further loops and increased parallelism is shown in Figs. 11 and 12, and in the latter figure the commencement of a secondary series of loops in the right free portion of the chain is seen at Y. In Fig. 13 a complete young colony is illustrated. It consists of a single chain of closely approximated bacilli, extending freely as a slightly wavy thread over the surface of the medium at both ends. The centre of the chain is much twisted and has formed numerous loops, and bands of parallel bacilli. On the free portion of the chain extending to the right a secondary system of loops (Y) has developed. The chain is incompletely broken at four points (A, C, D and E). These incomplete breaks are no doubt due to partial ruptures of the capsule, for it seldom happens that the chain becomes completely divided at such places. The appearances seen at one of these points is illustrated on a larger scale at B, B[1].

In *Plate III* a series of photographs (× 240) are reproduced illustrating the formation of a superficial colony from two anthrax bacilli lying close together.

In Fig. 1 a long bacillus is shown with an abrupt angle near its centre. To the left of the angle and above the first bacillus lies the much shorter second bacillus. Fig. 2 (35 minutes) shows an increase in the length of both bacilli, and accentuation of the angle to such a degree that the portions of the bacillus forming it are almost parallel. Fig. 3 (120 minutes) shows a great increase in the length of the chain. That portion which originally formed the angle has grown out into a long curved process, composed of two parallel threads. The early formation of loops in the central portion of the chain is also seen. On the portion of the chain extending to the right a small angle may be noticed. The smaller bacillus is partly following the curves of the larger. Fig. 4 (170 minutes) shows increased growth and increased loop formation. The angle noticed on the right portion of the chain has now developed into a process and a loop is in the process of formation. Fig. 5 (215 minutes) illustrates a further advance in loop formation and the development of parallel strands of bacilli. Both the right and the left ends of the chain are now seen to be producing secondary systems of loops. Fig. 6 (292 minutes) illustrates a more advanced condition of the central portion of the colony. Parallelism is well marked and numerous loops are seen. The rapidly developing secondary system of loops on the left end of the chain is well shown. Fig. 7 (× 100) is a photograph of the complete colony after seven hours' growth. The primary system of loops is well shown in the middle and close to it on each side the secondary systems formed on the two ends of the chain. The two free ends are seen extending downwards. Above each of these is a less curved apparently freely projecting thread. These are in reality processes composed of parallel threads of bacilli produced in the same way as the process in Fig. 3. Fig. 8 illustrates the same colony after 23 hours' cultivation. Growth has mainly occurred in the central portions of the colony resulting in the production of very numerous loops, and large numbers of strands of parallel threads of bacilli. Moreover the whole colony is not now on the same plane as increased growth in the centre has caused some strands to override others.

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[1] In order to avoid confusion the segmentation is omitted in the later figures, 11 to 13. The continuation of the left free portion of the chain is illustrated in the detached figure below the colony.

In this series of photographs the formation of a complete colony is illustrated almost from the commencement of growth to the formation of a typical anthrax colony consisting of a raised centre of wavy strands surrounded by numerous loops and a few projecting threads.

The development of such a colony has been followed on many occasions and complete division of the original chain has very seldom been noticed. Occasionally, however, this does occur after an acute angle has been formed. It may therefore be stated as a general rule that a superficial anthrax colony consists of a single twisted chain of bacilli.

In the depth of the agar as on the surface a chain of closely approximated bacilli is first produced. As the chain grows it becomes irregularly curved; but owing to the resistance of the agar regular loops cannot be produced, and instead distorted loops, which may be more accurately described as lateral outgrowths composed of spirally twisted threads, are thrust out into the medium. At other places the attempt at loop formation results in the chain becoming much twisted on itself, and gives rise to the appearance of knots on the chain. Finally the colony consists either of a single knot or a series of knot-like masses, surrounded by wavy outgrowths composed of spirally twisted chains of bacilli.

Parallelism such as is seen in superficial colonies never occurs in deep colonies. In *Plate IV* (Figs. 1 to 10) a series of photographs (× about 80) are reproduced illustrating the formation of a deep colony from a single anthrax bacillus. In Fig. 1 a long wavy chain is seen, whose development from a single bacillus has been followed. Fig. 2 (15 minutes) shows an increase in length and a marked irregularity in the chain some little distance to the left of the centre. In Fig. 3 (45 minutes) the irregular area previously seen has become more distinct. In Fig. 4 (80 minutes) two small processes, just beginning to develop, can be made out one on each side of the irregular area and some distance from it. This figure also shows projecting processes one on each side of the irregular area. In Figs. 5 and 6 (117 and 135 minutes) the development of these processes may be followed. In Fig. 6 the irregular area now resembles a knot. Figs. 7, 8 and 9 (165, 210 and 285 minutes) illustrate further development. Fig. 10 (450 minutes) shows the fully developed colony. The irregular area first noticed has developed into a large knot, and several smaller knotted masses, produced in the same way, are seen along the length of the colony. Many irregular processes project laterally from the colony, some of which have developed knots in their length. At each end the colony terminates in a wavy thread.

In Figs. 6 to 10 another colony can be followed developing above the original colony, and in Figs. 7 to 10 a third colony developing below it.

Owing to their uneven disposition photographs of deep colonies have to be taken at such a low magnification that the details of the development of knots cannot be followed. While the photographs just described were being taken drawings were made at the same time on a larger scale. Diagram 2 reproduces a series of drawings showing the development of the irregular area from the condition seen in Plate IV, Fig. 2 to the condition seen in Plate IV, Fig. 5.

In *Diagram* 2, Fig. 1 (15 minutes) the structure of the irregular area is clearly shown. It is caused by several definite loops in the course of the chain, at two of which (A and B) the chain is sharply twisted. Figs. 2 and 3 (25 and 35 minutes) show the further development of the loops. In Fig. 4 (45 minutes) continued development has resulted in the formation of a loose tangle. The knot resulting from the further development of the tangle is seen in Fig. 5 (117 minutes). The gradual intertwining of the thread composing the tangle results in the formation of a tight knot at first slightly smaller than the original tangle. The two twisted loops, A and B, seen in Fig. 1 have developed into twisted processes, A_1 and B_1. Two other lateral processes C and D arising directly from the original chain are also seen.

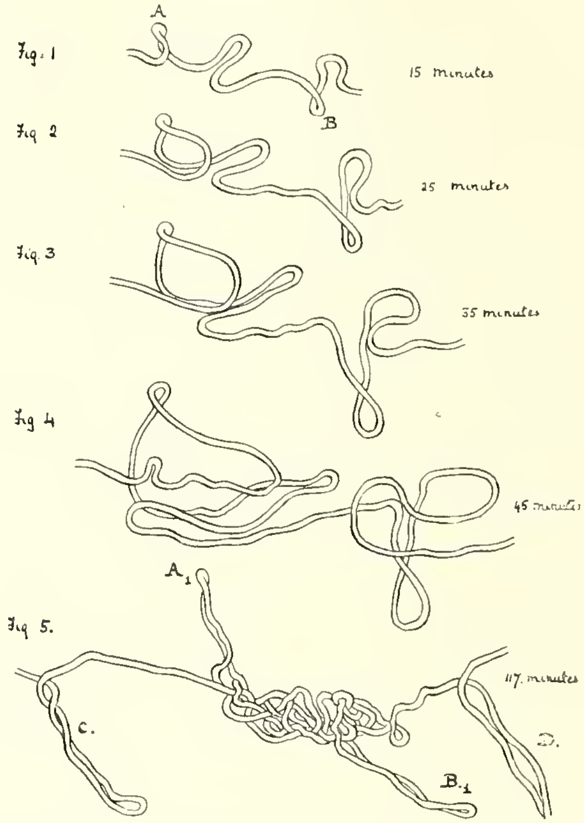

Diagram 2. Illustrating the development of *B. anthracis* in the depth of agar.

The lateral outgrowths of the deep colonies of *B. anthracis* are all due to such spiral or twisted outgrowths from the chain. These processes occasionally develop tangles or knots at various places in their length. In very rare cases only has a complete rupture of the original chain been seen.

Plate IV, Fig. 11 illustrates a typical deep anthrax colony in agar. It consists of a dense central mass with numerous more or less radial outgrowths, in most of which knots have been formed at various places, and all of which possess secondary lateral processes.

Plate IV, Fig. 12 illustrates some young deep anthrax colonies. These consist of central knots and numerous wavy radiating processes. This preparation was made with very dilute agar which limits the growth to a much smaller degree than does ordinary agar.

Plate IV, Fig. 13 shows deep and superficial growth in the same colony. The colony first developed just below the surface of the agar and grew in the typical manner. This portion is seen in the lower left hand part of the photograph. Later a portion of the colony reached the surface and produced on it a typical superficial wavy growth seen in the upper and right hand portions of the photograph.

In fluid media long chains occur, but owing to their growth being unrestricted they produce neither loops nor tangles.

The conditions seen in stained preparations and in "contact" preparations and the observations on living specimens just described all tend to show that in the "loop forming" group, as illustrated by *B. anthracis*, although the protoplasm becomes segmented and numerous bacilli are produced, the membrane or capsule surrounding the chain apparently remains continuous, and keeps the newly formed bacilli closely connected with one another. This capsule is seldom completely ruptured, and each colony therefore consists of a single uninterrupted chain of bacilli. Under the conditions of observation, or on surface cultures, the bacilli are only free to grow horizontally. As growth is taking place, not only at the ends, but in all parts of the chain at the same time, curves ultimately developing into loops are formed in order to accommodate the rapidly increasing length of the chain.

If the centre of a piece of string is placed between two parallel glass plates, separated from each other by a distance equal to the diameter of the string, and the string is gradually pushed between the plates from fixed points on each side, the changes which occur in a young colony of a loop forming bacillus are to some extent reproduced.

In the depth loop formation is hindered by the consistency of the medium and consequently the chain as it grows tends to develop tangles, which subsequently become knots, and twisted lateral outgrowths.

Group II. The "folding" group.

During the first stages of growth on the surface the original bacillus grows in length and divides into two rods of equal length, separated from each other by a very distinct interval. By further growth a straight or slightly curved chain composed of three, four or more distinct rods is produced. As growth progresses and more rods are formed the chain no longer remains straight or slightly curved, but develops angles at the points of junction of the various bacilli. These angles rapidly become more and more marked so that the chain exhibits a folded or zigzag appearance. By the further development of the folding process many of the rods come to lie parallel with each other, often with their long axes at right angles to the direction of the original chain. Continued growth then gives rise to the development of strands of parallel chains of bacilli, which ultimately become curved and wavy. Irregularities in the folding process not infrequently produce distorted loops.

The development of a bacillus belonging to this group on the surface of agar is illustrated in *Plate V.* Fig. 1 (× 250) shows a single, slightly curved bacillus. At its lower end a thinner pale prolongation is seen. This either represents an empty portion of the capsule or perhaps a dead bacillus. It is evidently incapable of growth and can be traced in exactly the same condition for 170 minutes (Figs. 1 to 6). Fig. 2 (75 minutes) shows an increase in length. Three bacilli are now seen, the lower two forming a slight angle with each other, and the third joined to the upper of the two at a greater angle by a faint connection, like the prolongation seen in Fig. 1. As in the former case no growth occurs in this region, which can be traced up to Fig. 6. The fact that its diameter greatly diminishes (Figs. 4 to 6) seems to confirm the view that it represents a stretched portion of capsule. In Fig. 3 (95 minutes) further growth has occurred, the two adjacent bacilli having divided into four, which lie at a small angle with each other, and the upper one having divided and formed a chain of two individuals, whose long axis is now disposed nearly at right angles to that of the main chain. Fig. 4 (125 minutes) shows the commencement of the folding process. The main chain has divided into seven individuals, and the angles formed at the junctions of the upper three and the lower three are very marked. In Fig. 5 (150 minutes) the main chain consists of thirteen individuals. The parallel arrangement produced in its upper and lower portions by the continuation of the folding process, resulting in the complete approximation of the bacilli forming the angles, is well seen. In Fig. 6 (170 minutes) the increase in the number of the bacilli forming the chain is well shown, and parallelism is a marked feature. This figure also illustrates the formation of angular loops, which are marked features in the peripheral portions of some colonies of organisms belonging to this group.

Such loops are produced by a modification of the folding arrangement, which is well exemplified in Figs. 4, 5 and 6. As the result of the approximation of the bacilli forming the marked angle at the lower portion of the chain (Fig. 4) a lateral process composed of two parallel bacilli is produced in Fig. 5. In the course of further growth one of the organisms forming this process grows faster than the other and the chain formed from it develops a secondary angle. The result is an irregular loop. Figs. 7 and 8 (× 150; 195 and 243 minutes) show the results of further growth. In the latter it is seen that the parallel chains of bacilli, well shown in Fig. 6, tend to develop considerable curves. In Fig. 7 a chain of bacilli from another colony has grown up and touched a prolongation of the colony under consideration at the left hand lower corner of the photograph.

In spite of the fact that the bacilli appear to have become separated at some of the angles, especially when these have become very acute, no separation really occurs in the early stages of growth. If such a chain as that shown in Fig. 5 is disturbed by a current of water its shape becomes completely altered, but it floats away without losing its continuity. In the later stages of growth complete ruptures are occasionally seen.

Fig. 10 (× about 30) shows the margin of a fully developed colony with a thin expansion composed of numerous angular loops.

Deep colonies show a modification of the process just described. The formation of one is illustrated in *Diagram* 3.

In Fig. 1 a chain with two angles and several curves is shown. Five minutes later (Fig. 2) both the curves and the angles have become better marked. During subsequent growth numerous angles are developed, each of which becomes more acute and ultimately results in a process, composed of two parallel bacilli, united at their distal ends, growing out in a direction more or less at right angles to the direction of the original chain. Most of the curves also sooner or later develop into angles. The lateral processes at a later period produce secondary lateral processes of their own in the same way. Although the parallel bacilli forming the lateral outgrowths are at first united at their distal ends, there is a tendency for the uniting portion of the capsule to rupture after the outgrowth has reached a considerable size, probably due to unequal growth in the chains formed from the two original bacilli producing the outgrowth. This is clearly seen in the outgrowth produced from the well marked angle in the centre of the chain in Fig. 1, and also in that produced from the large curve near the bottom of this figure.

In the figures the formation of various angles and the subsequent outgrowths can be clearly followed up to 135 minutes (Fig. 10). Later development becomes irregular and often results in the production of knot-like masses in the main chain, as may be seen in Figs. 11 and 12, representing the upper portion of the colony after 210 and 310 minutes growth respectively. Fig. 11 represents a later stage of the upper portion of the colony above the point A, and Fig. 12 a later stage in the development of Fig. 11 above the point B.

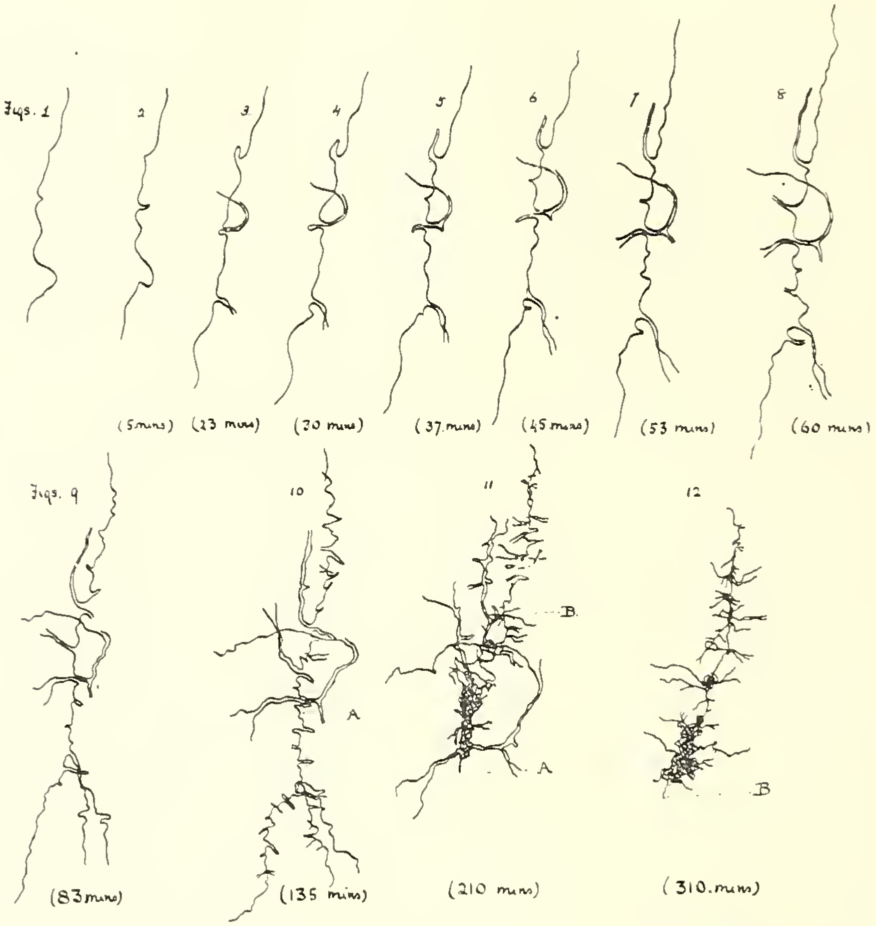

Diagram 3. Illustrating the development of a "folding" organism in the depth of agar.

Plate V, Fig. 9 illustrates the upper portion of this colony at a still later stage (6 hours).

Three organisms, morphologically resembling *B. anthracis*, belonging to this group have been investigated and all of them produce, in spite of the difference in the mode of development, colonies resembling those of *B. anthracis*. These colonies consist of twisted strands of parallel bacilli occasionally surrounded by fringes of irregular loops (Plate V, Fig. 10). The central portion of the colony has been well described as looking under a low power lens like a mass of cracked ice.

The peculiar folding which is so noticeable during the early development of the colony is probably made possible by the length and flexibility of the parts of the capsule connecting the individual bacilli composing the chain. The length of this connecting portion of the

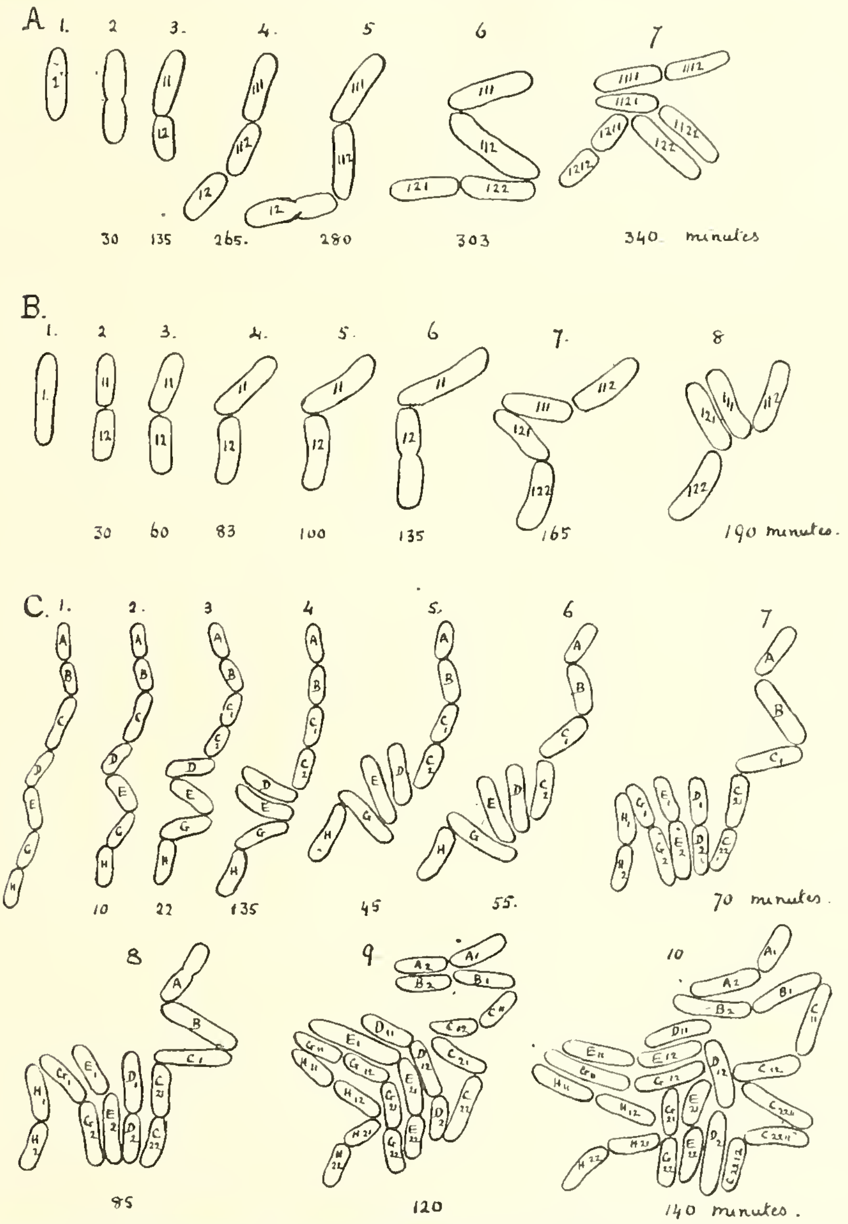
A
1. 2 3. 4. 5 6 7
1' 11 12 111 112 121 122 1111 1112 1121 1122 1211 1212
30 135 265. 280 303 340 minutes
B.
1. 2 3. 4. 5. 6 7. 8
1. 11 12 111 112 121 122
30 60 83 100 135 165 190 minutes.
C. 1. 2. 3 4 5. 6 7
A B C C1 C2 D E G H
10 22 135 45 55. 70 minutes.
8 9 10
A1 A2 B1 B2 C11 C21 C22 D1 D11 D12 D2 E1 E11 E12 E2 E21 E22 G1 G11 G12 G2 G21 G22 H1 H11 H12 H2 H21 H22
85 120 140 minutes.

Diagram 4. Illustrating the development of *B. pestis* on the surface of agar.

capsule can be readily demonstrated both in stained and living preparations (see Plate V, Fig. 6), in each case distinct, relatively long, intervals being seen between the bacilli composing the chain. Its flexibility can also be easily demonstrated in young living preparations by running successive drops of water between the agar and the cover-glass. The currents which are set up cause the chain to bend in various directions, with the production of varying angles at the junctions of the bacilli.

The post-fission folding movements seen in this group can be reproduced by replacing the string used to imitate the changes in the loop forming group by a chain with long links, or by a series of tubes loosely connected by a string run through them.

B. pestis, which appears to be totally unrelated to the other members of this group, also frequently exhibits folding post-fission movements in the formation of superficial colonies. Diagram 4, series A and B, illustrate early folding movements in short chains developing from single bacilli, and series C the folding movements in a long chain of seven individuals.

Group III. The "snapping" group.

The mode of division and the curious post-fission movements peculiar to this group were first briefly described by Kurth (1898) and later by Nakanishi (1901). Hill (1901, p. 81) however was the first to thoroughly investigate the subject in living specimens growing on the surface of agar, and to give a detailed description of the process. He was also the first to clearly differentiate the "snapping" type of post-fission movement seen in this group from the "slipping" type found in the next group. Hill and Rickards (1903) continuing these investigations only found snapping post-fission movements in *B. diphtheriae, B. pseudo-diphtheriae* and *B. xerosis.*

The writer, during the course of the present investigations, has made observations on a number of diphtheroid organisms obtained from various sources. In all these snapping post-fission movements occurred, but it was never seen in other types of bacilli.

The mode of post-fission movement may therefore be regarded as characteristic of the diphtheroid group.

"A gradual enlargement of the bacterial cell in both diameters accompanied often by slight changes in outline suggesting some plasticity of the bacterial walls" is the first change seen when a single living bacillus is carefully examined. "At times it may be possible to detect segmentation of the protoplasm previous to the post-fission movements, a hard dark line, not necessarily central, developing across the axis of the rod; sometimes more than one such segmentation line is visible in a single rod." Usually however no such changes can be made out and without warning there is a sudden snapping across of the rod resulting in the two portions lying at an angle with each other. "Subsequent growth results in the gradual approximation of the distal ends of these two rods to each other giving a more or less parallel arrangement. The proximal ends of the new rods (those which before snapping were continuous with each other) remain contiguous to each other after parallelism is achieved. In such movements one or both rods necessarily describe parts of a circle, and when, as often happens, the two rods are not of the same length, the shorter usually moves through a much greater arc than the longer. Subsequent growth does not usually result in the separation of the two portions at the proximal ends, the increase in length in the larger portion apparently carrying the other portion with it. That something unites the proximal ends seems to be further evident because if two rods thus derived be disturbed, as by a current of water, so that they drift off, turning over and over, their relative positions remain unaltered. A single rod may break up into three portions instead of two."

After a period of further growth the two newly formed rods again divide in the same way. The continuation of this process gives rise to the peculiarly arranged Chinese letter like groups of bacilli so characteristic of the diphtheria group.

Plate VI, Figs. 1 to 8 (× 100) illustrate the changes undergone by three diphtheria bacilli. In the left hand specimen in figure 1 a single division has already taken place and the two new organisms are disposed at nearly a right angle to each other. The central organism has also just divided, while the right hand one has not. In Fig. 2 (55 minutes) the two bacilli on the left have again divided, producing a colony of four bacilli disposed at various angles to each other, and considerable growth has occurred in the central specimen. In Fig. 3 (95 minutes) further growth has occurred in the central and left hand specimens. In the latter the distal ends of the organisms formed during the last division are beginning to approach one another. The right hand specimen has now divided. In Figs. 4, 5 and 6 (125, 180 and 280 minutes) multiplication by similar divisions can be followed until three separate colonies are seen in Fig. 7 (315 minutes). The very considerable variations in size of the component bacilli are clearly shown. In Fig. 8 (370 minutes) the three colonies have coalesced.

During the earlier stages of growth such as are seen in Figs 1. to 5 "snapping" in one bacillus does not influence the others to any extent.

In the later stages however the disturbance caused by one organism snapping moves a number of others and alters the relative positions of the members of the group.

In Fig. 9 (× about 300) two curved bacilli are seen. The upper one subsequently undergoes no change, but the lower bacillus gives rise to a small colony. Fig. 10 (115 minutes) represents the condition seen shortly after the division of the lower bacillus, and Fig. 11 (130 minutes) illustrates the approximation of the distal ends of the new rods resulting from further growth. Fig. 12 (240 minutes) shows a small colony of seven individuals formed from the lower bacillus. The upper bacillus is straighter than in the earlier photographs, but this change is probably due to pressure on its lower end produced by contact with one of the newly formed organisms.

Fig. 13 illustrates the condition of a suitable field at an early stage of development (55 minutes after the observations commenced). Single bacilli or pairs of bacilli, developed from single individuals, are scattered over the field. Fig. 14 shows the same field after six hours' growth. Here seven large colonies are seen. Four (A, C, E, G) have

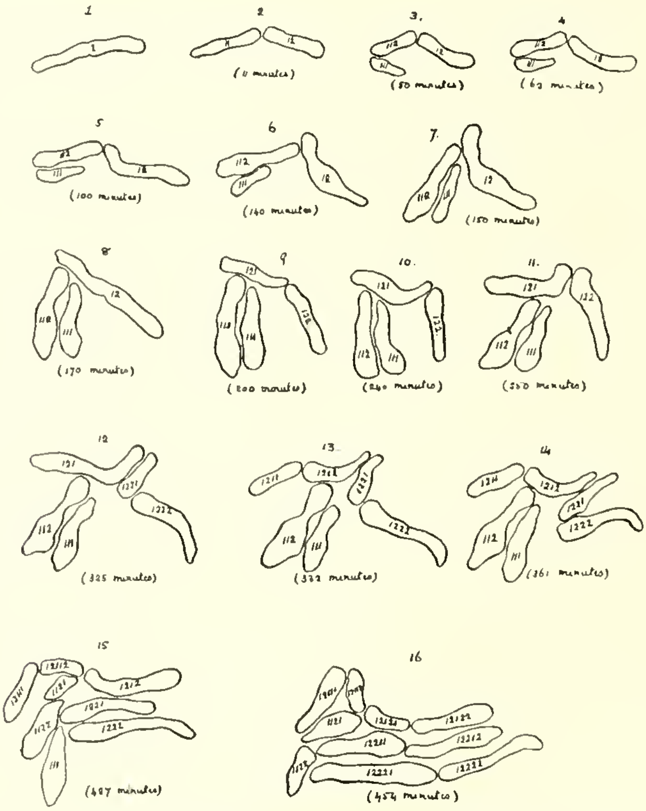

Diagram 5. Illustrating the development of *B. diphtheriae* on the surface of agar.

been developed from single bacilli (a, c, e, g Fig. 13), and three (B, D, F) by the fusion of the products of two or three originally separate bacilli (b_1, b_2; d_1, d_2; and f_1, f_2, f_3 respectively). The right hand colony (F) in this figure is the one whose growth has been followed in Figs. 1 to 8.

Diagram 5 illustrates the development of a colony from a single diphtheria bacillus. The tendency to the production of parallelism is specially marked in this series. During the course of growth one of the newly formed organisms (Fig. 3, No. 111) apparently degenerates. Although it grows large it never divides and later becomes pale and ill defined (Fig. 15) and eventually disappears (Fig. 16).

Diagram 6 also illustrates the development of a diphtheria colony. It differs from the last series in showing a number of giant and irregular forms (Fig. 7, No. 31; Fig. 8, No. 32; Fig. 12, No. 21) most of which subsequently divide. One curious wedge-shaped organism (Fig. 8, No. 313) undergoes no further development. This series also illustrates the division of one bacillus (Fig. 7, No. 31) into three (Fig. 8, Nos. 311, 312 and 313).

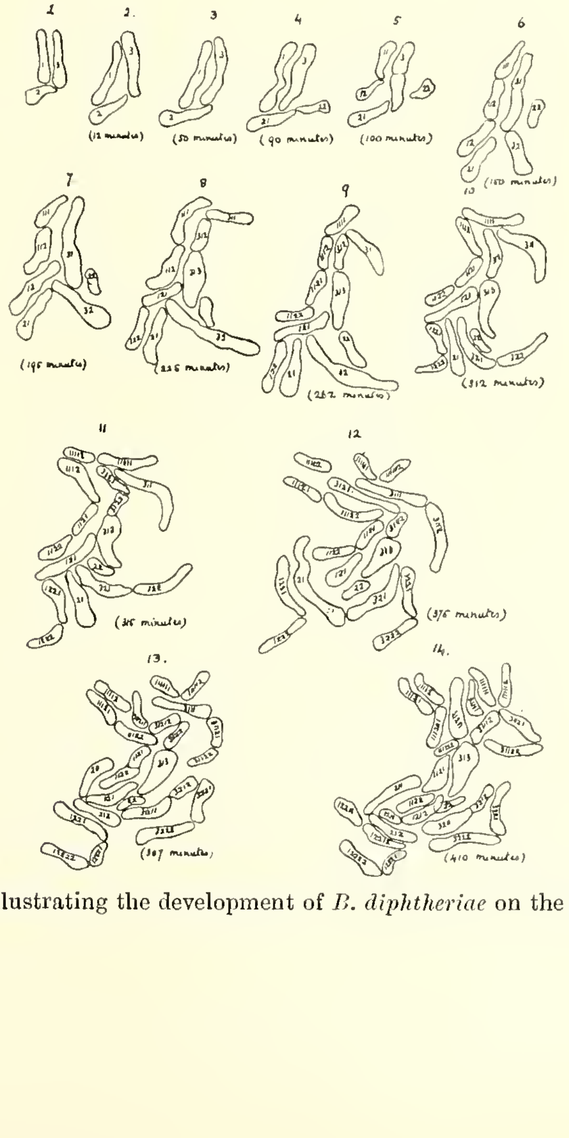

Diagram 6. Illustrating the development of *B. diphtheriae* on the surface of agar.

Diagrams 7 *and* 8 illustrate the same type of division and post-fission movement in eight diphtheroid organisms, (A) *B. Hofmanni*, (B) *B. coryzae segmentosus*, (C) *B. xerosis*, (D) a diphtheria-like bacillus from the eye of a diseased turkey, (E) a bacillus from a plate exposed to the air, (F and G) two different species from the mouth and (H) one from the lung of a grouse.

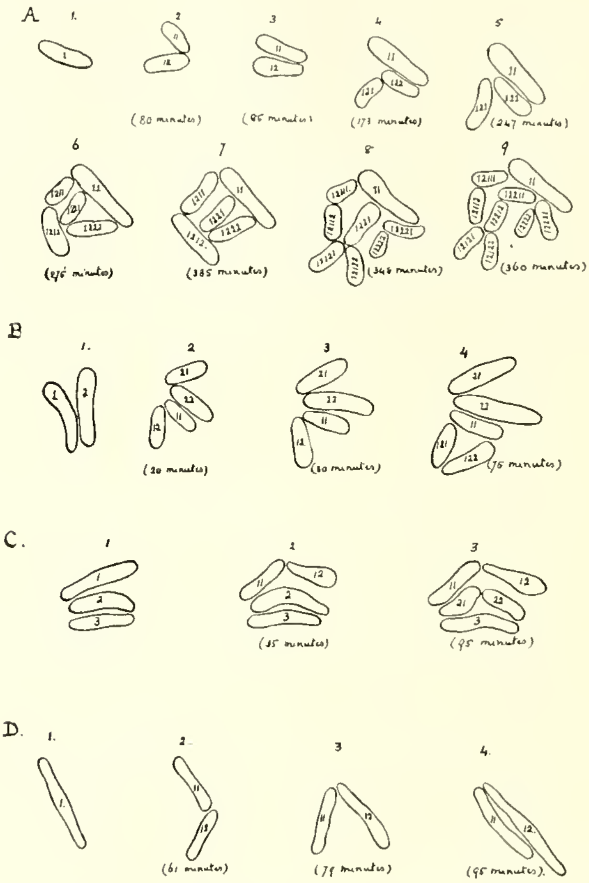

Diagram 7. Illustrating the development of various diphtheroid organisms on the surface of agar.

Diagram 7, series A, has been selected for illustration because it shows the mode of formation of the so-called "pseudo-diphtheria" type of *B. Hofmanni* (Figs. 4 to 9, No. 11, and Fig. 7, No. 1212).

In each figure the time which had elapsed from the beginning of the observation is indicated. By reference to these figures it will be seen that some organisms divide very quickly and others very slowly.

Similar snapping post-fission movements occur in deep growths, but the resulting bacilli remain more nearly in line than they do on surface growths. As growth continues the distal ends of the newly formed bacilli very gradually approach each other, but the organisms seldom become parallel.

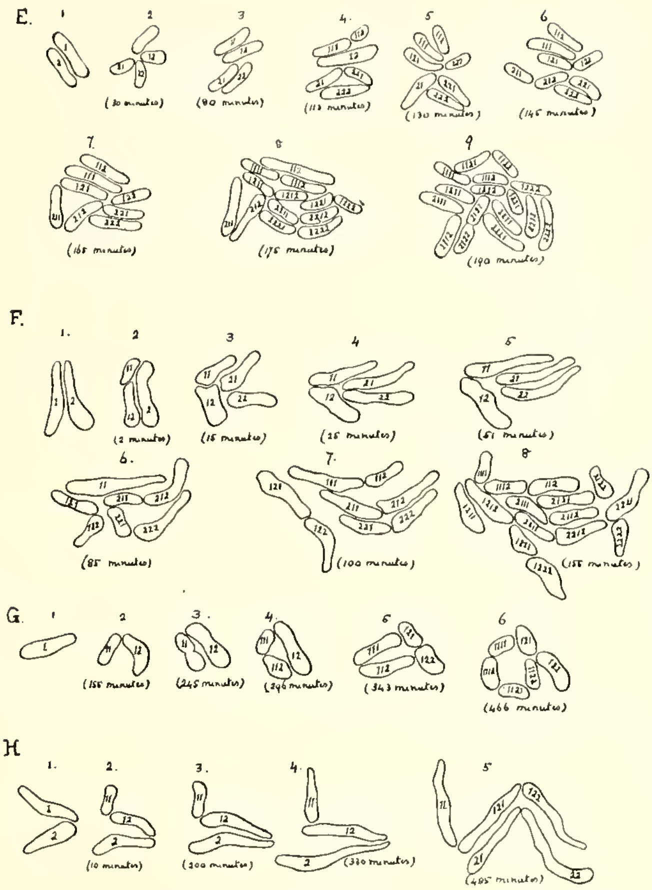

Diagram 8. Illustrating the development of various diphtheroid organisms on the surface of agar.

Hill (1901, p. 81) offers the following hypothesis in explanation of these movements. "That the visible bacterial rod is surrounded by an invisible or scarcely visible membrane; that in *B. diphtheriae* (and other members of this group) only incomplete rupture of this membrane after fission occurs, the line of rupture running round only a portion of the circumference of the original rod, leaving a bridge connecting the new rods formed; that the enlargement of these rods before snapping and the tension thus produced originate the rupture; that the snapping is due to the sudden occurrence of the rupture, and that the preliminary angular position and the parallelism finally achieved are due to the pressure of the proximal ends of the new rods on each other, in composition with the restraint exercised by the unruptured strap-hinge-like portion of the original membrane."

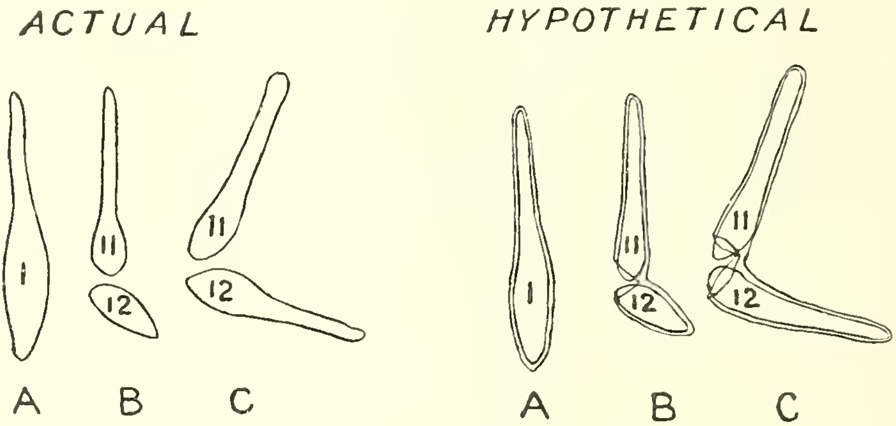

Diagram 9.

Diagram 9 is a reproduction of Hill's figure illustrating his hypothesis. "Fig. 1.—*Actual. B. diphtheriae,* illustrating 'snapping.' (A) became (B) suddenly, the change occurring under the writer's eye; Fig. 1.—*Hypothetical. B. diphtheriae,* illustrating the writer's hypothesis of membrane rupture, devised to account for the snapping and subsequently achieved parallelism."

Group IV. The "slipping" group.

Hill (1901, p. 81) seems to have been the first to accurately describe and illustrate by means of a diagram the post-fission movements of this group, calling them "slipping" movements.

The writer has found that *B. typhosus, B. enteritidis* (Gaertner), *B. coli, B. pneumoniae* (Friedländer) and allied organisms, *B. pyocyaneus,* the butter bacillus of Rabinowitch, *V. cholerae, S. rubrum* and other vibrios, *B. fluorescens, B. subtilis* and allied organisms and many other species of non-pathogenic bacilli belong to this group.

The characteristic mode of development on the surface of agar is as follows. The original single rod grows in length and divides, "the fission being always clearly evidenced by a sharp separation of the protoplasm of the original rod into two usually equal portions, by a translucent segmentation interval. Later a slight curve to one side is observed in one of the two new rods still in line with each other" or both rods become slightly bent, though one is usually more bent than the other. "This curve tends to straighten afterwards, the straightening being achieved by the proximal end of the curved rod slipping slowly out of line with the other rod, towards that side on which was the convexity of the curved rod. The two rods now free at both ends continue to grow, the proximal ends thus passing each other in opposite directions, and finally reaching, if nothing prevents, to the distal ends." This process is repeated again and again till a young colony composed of a large number of parallel bacilli is produced.

Plate VII (× 625) illustrates the formation of such a colony in the case of an organism of the *subtilis* group.

Fig. 1 shows two bacilli end to end, with a clear segmentation interval dividing them. The organism to the left is slightly bent with the convexity upwards. In Fig. 2 (45 minutes) division has taken place in the organism on the left, but all are still in line. In Fig. 3 (55 minutes) the organism on the right is beginning to slip past the

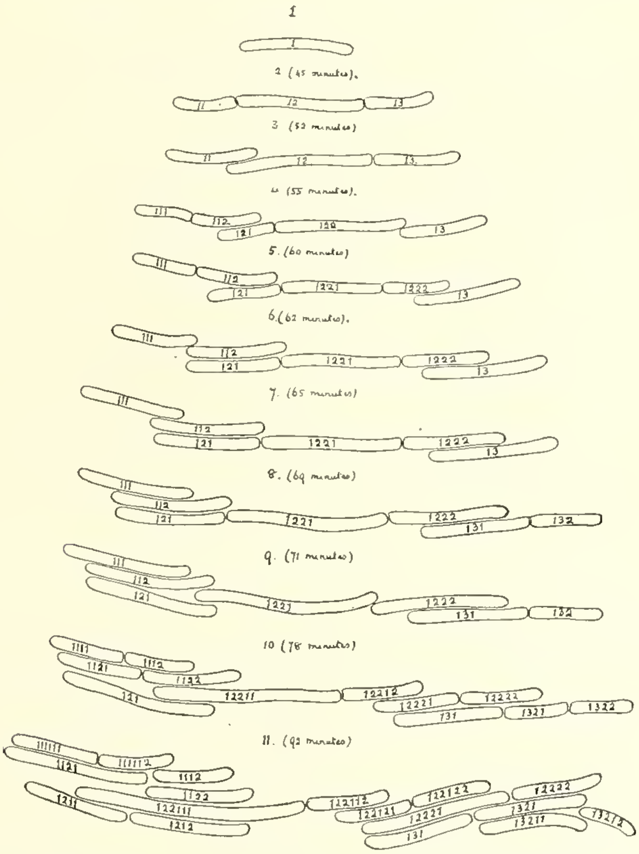

Diagram 10. Illustrating the development of *B. coli* on the surface of agar.

central organism. Figs. 4 and 5 (70 and 90 minutes) show the continuation of this process. In Fig. 6 (105 minutes) the organism to the left is slipping past the central one and in Fig. 7 (120 minutes) the condition is more advanced. At this stage the colony consists of three parallel lines of bacilli. The further development of this colony by the same methods is illustrated in Figs. 8 to 11 (140, 180, 225, 250 minutes respectively). In the latter figure the colony consists of 109 distinctly separated rods and two long unsegmented threads which become parallel with each other at the right hand extremity of the colony. In Fig. 12 (340 minutes) the colony is just beginning to fuse with another colony at the bottom right hand corner of the photograph.

During all this time the bacilli forming the colony remained in one plane.

Diagram 10 illustrates the development of a young colony of *B. coli* from a single cell, and *diagram* 11, series A and B, the development of *V. cholerae*. Series B, Fig. 13 shows some very long forms.

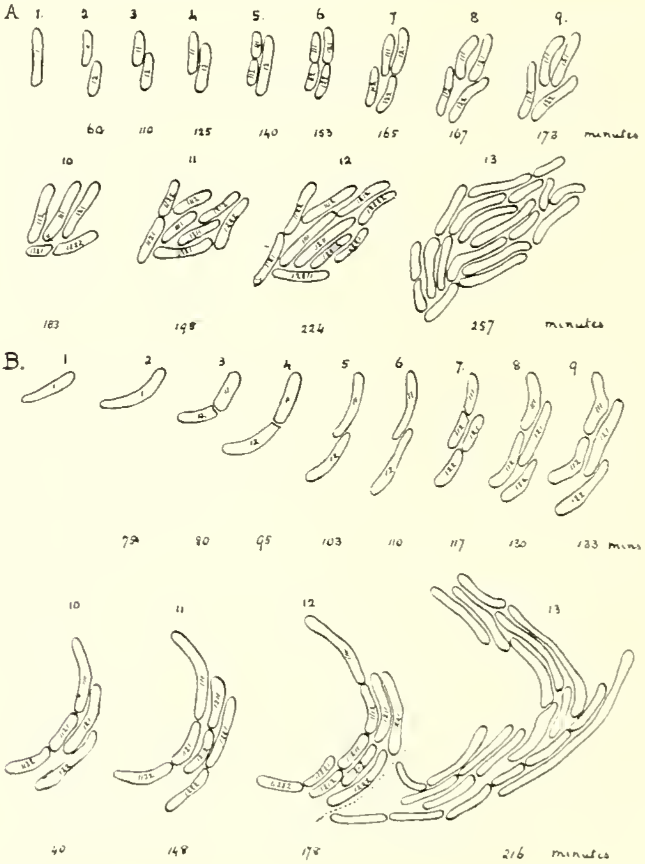

Diagram 11. Illustrating the development of *V. cholerae* on the surface of agar.

Irregularities due to extreme rapidity of growth or to the formation of long unsegmented threads may occur, but are seldom met with in cultures of the smaller bacilli belonging to this group. In the former case "the rods may grow so fast after separation that instead of ultimately lying side by side the impinging of one upon the other near the proximal end of the latter carries this latter in front of it, and swings it round so that it lies right across and in front of the axis of the first, forming an irregular T." Irregularities due to this cause are not very common in surface cultures. A more common and more important cause of irregularity of growth, which is more especially apt to be a disturbing factor in the case of large, relatively strong, bacilli of the *subtilis* group, is due to the formation of long unsegmented threads. Very frequently the gradual increase in length in such threads causes them to develop primary and secondary loops, such as occur in surface cultures of *B. anthracis.* Ultimately however segmentation usually occurs and owing to the bacilli composing the loop slipping past each other an irregular network is produced.

Diagram 12, series A, illustrates the formation of long parallel partially segmented threads in a culture of an organism belonging to the *subtilis* group, and series B illustrates the changes which occur in a loop developed from such parallel threads. In series B, Fig. 1 a well formed loop is seen, and in Figs. 2, 3 and 4 the gradual development of secondary loops. In the latter figure however slipping has already commenced at *a.* In Fig. 5 the secondary loop on the left is still better marked, but the primary loop is beginning to break up by segmentation (b, b, b, b) of the threads composing it. Fig. 6 shows the irregular network resulting from the breaking up of the loop by segmentation and slipping.

This series also illustrates another irregular post-fission movement which is not infrequently seen in preparations of some strong film forming organisms. Instead of slipping past each other the ends of the newly divided bacilli press against each other (B, Fig. 1, X), and cause the proximal ends to be directed outwards at a considerable angle from the original chain (B, Fig. 2, X). As growth progresses a process consisting of two parallel bacilli is thus formed (B, Figs. 3, 4, 5, X).

In deep growths of organisms belonging to this group typical slipping post-fission movements follow division, but whether the deep colonies ultimately produced are small and more or less rounded or large and branched and "root-like" seems to depend on the capacity of the organisms producing them to overcome the resistance of the agar.

Strong fast growing organisms, such as *B. subtilis*, produce root-like colonies, while small, relatively weak organisms, such as *B. coli*, rounded colonies.

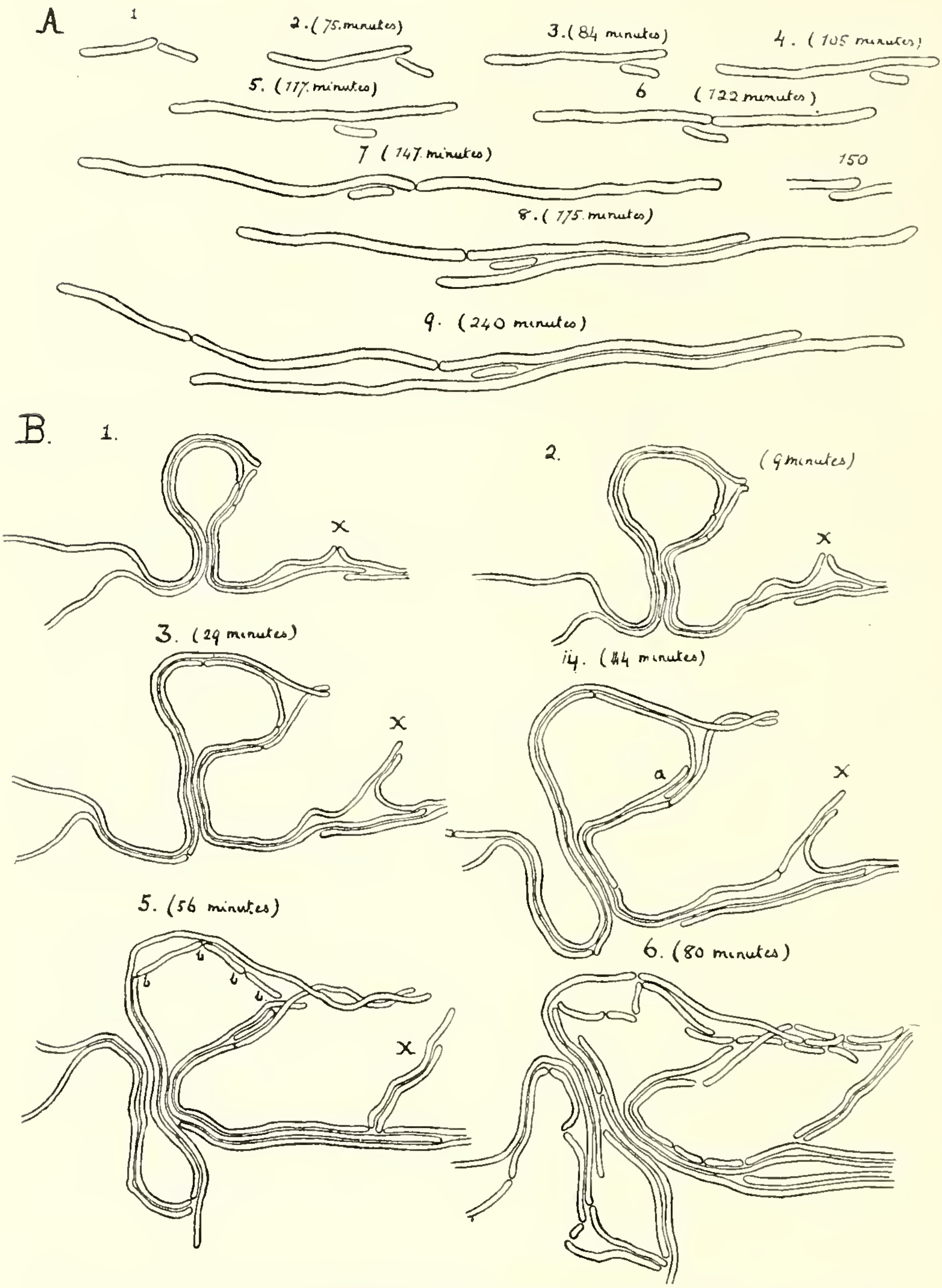

Diagram 12. Illustrating certain irregular modes of development in an organism of the *subtilis* group on the surface of agar.

Many of these small colonies are lens shaped, *i.e.* rounded in one plane and biconvex in the other, and are formed in the following way. By slipping post-fission movements a flat more or less rounded colony, such as that seen in Plate VIII, Fig. 4, is produced. As growth continues large numbers of new bacilli are produced, more especially in the central portions, and begin to override those already formed. The continuation of this process results in the central parts being ultimately composed of many layers and the peripheral parts of fewer layers of bacilli. Hence the biconvex shape on side view.

In the case of the stronger and more rapidly growing bacilli, however, the newly formed organisms in the early stages of growth tend to grow in various planes, and the colony consequently assumes a very irregular branched appearance. During the later stages of growth, when frequent divisions are occurring in the organisms composing the early branches, regular slipping post-fission movements with the formation of parallel strands of bacilli are seen. Possibly the earlier branches produce line of cleavage or areas of less resistance in the agar. The development of a deep colony of this type is illustrated in *Diagram* 13.

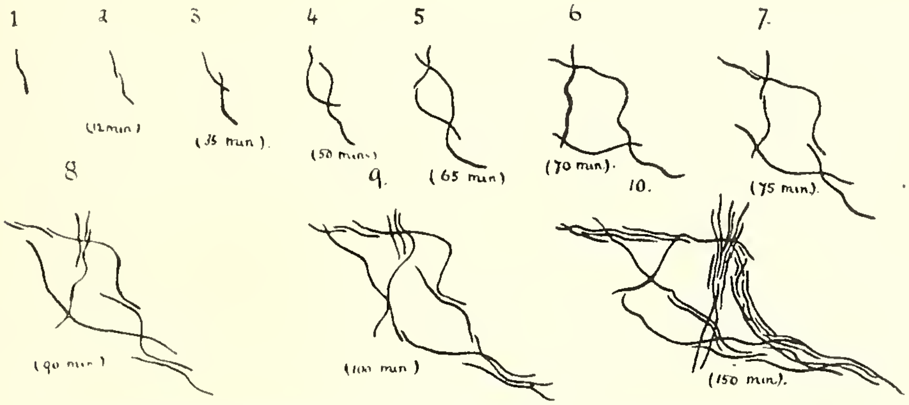

Diagram 13. Illustrating the development of an organism of the *subtilis* group in the depth of agar.

In fluid media cultures of organisms belonging to this group show free, single, usually motile individuals, since there is nothing to retain the rods in their relationship to one another. Pellicles composed of parallel bacilli are however common.

All the facts hitherto observed tend to confirm the hypothesis that in the "loop forming" group the structure of the colonies, superficial and deep, is due to the strength, close application and non-liability to rupture of the capsule covering the chains; in the "folding" group to its length and flexibility between the bacilli composing the chains; in the "snapping" group to its incomplete rupture during division and the persistence of this condition subsequently; and in the "slipping" group to its complete rupture at the time of division.

The fully formed colonies produced by certain members of the "folding" group may be at times somewhat difficult to distinguish from anthrax colonies. This is due to the fact that in both cases wavy strands composed of numerous parallel chains of bacilli compose the central portions of the colonies. Under a lens both consequently have a "cracked ice" appearance. Usually the margins of the colonies of the folding group are thick and wavy being formed of strands similar in structure to those which compose the centre. More rarely a thin expansion of irregular loops may be seen (Plate V, Fig. 10). On the other hand anthrax colonies almost invariably possess thinner margins, composed of well formed loops, and very frequently exhibit outgrowths of the type shown in Plate IV, Fig. 13. The terminal portions of the chain, with their secondary systems of loops (Plate III, Fig. 7), may also often be found.

More rarely colonies, somewhat resembling anthrax colonies, may be produced by members of the *subtilis* group (slipping group). These are only formed when long unsegmented filaments are being produced, which necessarily behave for mechanical reasons in the same way as anthrax chains (see Diagram 14).

The colonies of bacilli.

During the progress of the observations which have just been described, some attempts were made to ascertain to what extent the mode of "post-fission" movement influenced the appearance of surface colonies, under ordinary conditions of cultivation. For the purposes of diagnosis and investigation considerable importance is necessarily attached to the appearance of surface colonies, owing to the aid it renders in picking out the suspected organisms in mixed cultures. It is of interest therefore to know to what extent the colonies produced by a given organism are capable of variation. Making use of the same batch of agar under constant conditions of incubation the factor which was found to influence to the greatest degree the forms assumed by the colonies was the dryness of the medium.

The first experiments were made with surface cultures on shallow layers of agar in Petri dishes. In the case of some organisms of the "slipping" group great differences were noticed in the types of the colonies formed on different parts of the dish. On searching for a reason of this phenomenon it was noticed that where the layer of agar was thicker owing to a depression on the bottom of the dish a certain type of colony developed, and where it was thinner another type. The different rates at which layers of various depths dried seemed to afford the most satisfactory explanation of the variations which had been noted. Owing to the uneven construction of these dishes the attempt to follow up these observations in them was abandoned and ultimately the following method was adopted.

Thoroughly clean, sterile glass plates, made from old quarter plate negative glasses, were placed on the bench in a slightly tilted position so that one end was about 0·3 to 0·4 cms. higher than the other. Melted agar was poured on to the plate near the upper end and allowed to run down nearly to the other end. In order to secure an even layer it was distributed with a hot, sterile platinum needle. Several plates were made at a time in this way and allowed to solidify and dry under bell jars for various periods of time. Finally the emulsion of the organism to be investigated was applied usually in three or more parallel longitudinal streaks with a thin platinum loop. The plates, separated by supports, were then arranged horizontally one above the other in a large glass Petri dish. A capsule of water was put into the dish, the lid put on, and the whole incubated at 37° C.

In the "loop forming" group no important differences in the colonies produced on the thick and thin portions of the agar were noticed in thinly sown cultures. Usually the colonies were larger and more inclined to spread at the thicker end, but the general type remained the same. When thickly sown the colonies coalesced to form a continuous film in the thicker portions. Even in continuous growths however indications of the original colonies could be discerned. In the thinner parts the colonies were however separate. In all cases the edges of the growth, whether in the form of a continuous film or as separate colonies, showed the characteristic regularly looped condition, and extensions, such as are illustrated in Plate IV, Fig. 13, were common.

The "folding" group behaved in the same way. The margins of the growths were however usually formed by thick wavy strands composed of parallel bundles of bacilli, and thin expansions of irregular loops, as shown in Plate V, Fig. 10, were uncommon.

In both these groups it is evident from the mode of post-fission movement that widely separated bacilli tend to produce separate, more or less rounded colonies, and that more closely aggregated bacilli tend to produce separate colonies whose peripheral portions fuse with each other, resulting in a slightly uneven continuous film. Such fusion only occurs on moist agar which allows of growth over its surface for a considerable time.

In the "snapping" or diphtheroid group continuous growth was never obtained unless the cultures had been very copiously sown, and in any case numerous elevations in such a growth marked the situations of the originally discrete colonies which had partially coalesced to form this film.

In the "slipping" group, however, the structure and appearance of the colony is very markedly influenced by the condition of the medium. The variations are so great that the colonies in different parts of the culture look as if they had been produced by different species of organisms. To illustrate the range of variation in a single species a member of the *subtilis* group has been chosen.

Plate VIII, Fig. 9 is a photograph of a plate sown shortly after solidification in two parallel streaks with an emulsion of this organism, and incubated for 18 hours. The thicker portion is at the lower end. Over the lower portion a uniform film is seen. (If further incubated folds are often produced on the film, which are due to small areas of the film being thrust away from the surface of the agar by the increasing lateral pressure. Such folds are very commonly present in the lower two-thirds of agar slant cultures.) Towards the middle of the plate the film becomes less dense, and at the upper part discontinuous. Here branched lines of growth, each terminating in a slightly thickened expansion, are seen radiating from the needle tracks. Smaller radiating processes are seen all round the film, being least marked at its thicker portions.

Fig. 8 shows a similar culture on agar which has been dried for a short time after solidification. Here there is practically no film, but in the thicker (lower) parts radiating growths are seen round denser centres. In the thinner (upper) parts the colonies are mostly dense and round.

Though the exact causes of these variations have not been determined, numerous cultivations have shown that they constantly occur under given conditions, and the mode of formation of each variety has been ascertained.

Where the layer of agar is thick and moist a film tends to be formed. In a thickly sown plate each bacillus produces a colony in the manner illustrated in Plate VII, and by the fusion of these colonies a fairly even film results. If the plate is less thickly sown isolated colonies are formed from each bacillus. From these processes run out, and join with those from neighbouring colonies forming a network. The uncovered spaces are filled in by lateral growth from the fused processes. The exact condition ultimately reached depends however on the rate at which the surface of the agar dries, or is in some other way rendered unsuitable for the development of further growth over it. If this occurs before the spaces of the network are filled in a discontinuous film as shown in Plate VIII, Figs. 8 (lower part) and 9 (upper part) results.

Isolated colonies growing on partially dry agar sometimes assume beautifully regular star-shaped forms. Plate V, Fig. 11 (nat. size) shows an extremely fine example of a colony entirely consisting of branched processes, and Fig. 12 illustrates the same colony after a further period of 24 hours' incubation. Here it can be seen that very little lateral extension has taken place, but the processes have enlarged especially at their extreme ends, where large rounded masses of growth have been produced.

In the driest part of the agar the growth is most limited as lateral expansion is early arrested, and rounded or oval heaped up colonies are generally formed. For a certain time the condition of the agar allows of lateral growth, and during this period a flat colony is formed. Subsequent growth results in the bacilli becoming superimposed and a thick colony being produced. Growth under these conditions has been studied and is illustrated in Plate VIII, Figs. 1—4. In Fig. 1 an elongated colony consisting of parallel bacilli all lying in the same plane is seen. Fig. 2 shows further development. In Fig. 3 it is seen that continued multiplication has caused some of the bacilli in the centre of the colony to override others. In Fig. 4 the difference between the central and peripheral portions is still better marked. The central portion consists of at least two layers of bacilli and the parallel arrangement is lost. The peripheral portion still consists of a single layer of bacilli. At this stage a curious phenomenon was noticed, namely very rapid motility at intervals on the part of groups of bacilli lying in the peripheral parts of the colony. These movements often began and ended rather suddenly and the bacilli never passed beyond the original limits of the colony. The blurred appearance in the right hand peripheral part of the colony is due to active movement during the exposure of the plate and a smaller blurred portion on the other side is due to the same cause. On further incubation the colony only increased slightly in size, but continued multiplication of the bacilli led to the formation of several layers.

Plate VIII, Fig. 7 illustrates a fully formed colony of this type with a thicker centre and thinner margins.

After general surface expansion has been arrested by the drying of the surface of the medium and raised areas of growth of considerable size have been produced further incubation may lead to an attempt at extension in the form of thin twisted, often branched ramifications. Fig. 5 shows on the right a number of such surface ramifications and on the left some thinner twisted threads due to extension in the depth. The two dark masses in the upper right and left corners are rounded masses at the extreme ends of long lateral processes. Fig. 6 shows the rare condition of a small round colony developing branching ramifications.

On somewhat dry agar a great tendency towards the formation of long partially segmented threads, which are probably important factors in the production of colonies with spreading processes, has been frequently noticed. The ramifications just described are also developed from radiating threads which grow rapidly outwards from the colony. Later segmentation and multiplication occur in the threads and consequently thin processes consisting of parallel bacilli are formed along the lines of the original threads.

Another closely allied species, which exhibits a considerable tendency to form long partially segmented threads, behaves somewhat differently. On thin agar the colony after a few hours' growth usually consists of a bundle of parallel threads (Diagram 14, Figs. 1, 1 a) produced by typical slipping movements, as illustrated in Diagram 12, series A. After further growth the bundle has a wavy or sometimes looped appearance, and threads may usually be seen projecting from it in various directions (Diagram 14, Fig. 2). On dry agar further incubation only results in some increase in length and in waviness of the central bundle. On moist agar the central parallel bundle often becomes markedly looped and twisted, and the projecting threads also produce twisted and often looped processes (Diagram 14, Fig. 3). Even though many loops may be produced on the process (Fig. 4) the latter cannot be mistaken for the process of a true loop-forming organism, for in all cases careful observation shows distinct evidence of "slipping," and single threads projecting from the loops are of common occurrence. Segmentation of the threads forming the processes ultimately occurs (Fig. 5) and indications of the original loops are lost. At this stage the positions of the original loops are marked by bands of parallel bacilli often disposed at right angles to the axis of the process (Fig. 6). If the condition of the agar permits very rapid lateral growth from such processes often occurs at this time.

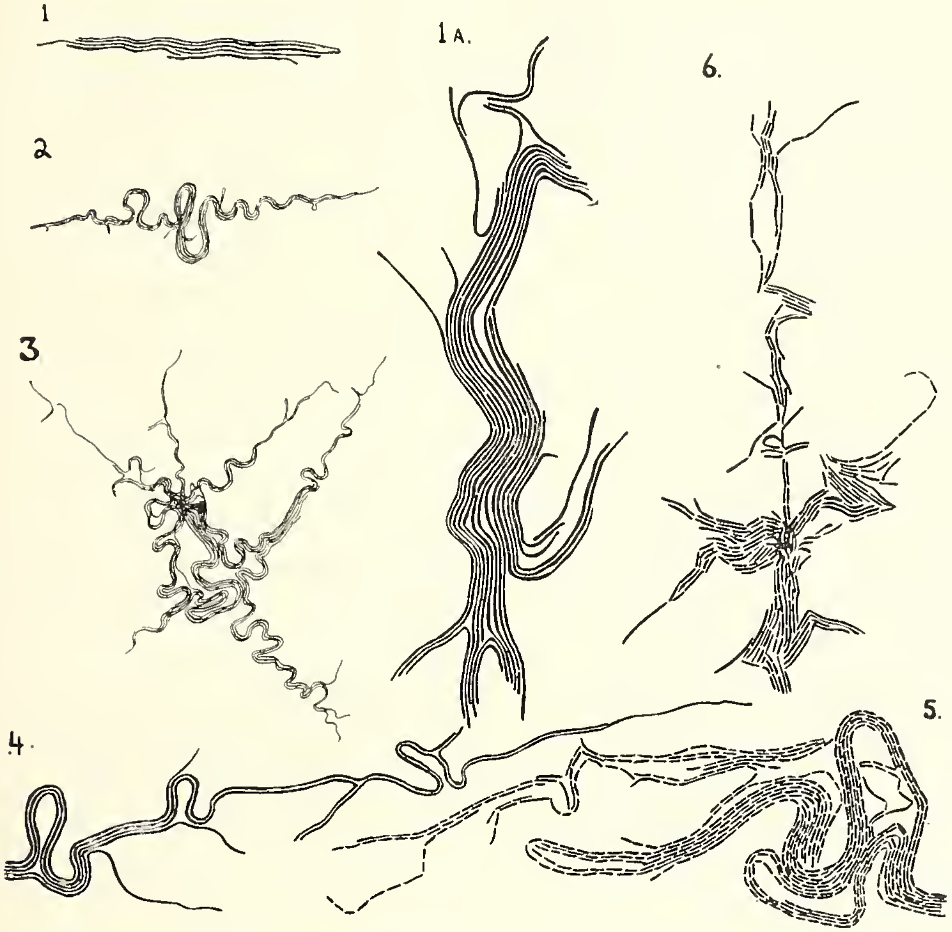

Diagram 14. Illustrating certain irregular modes of development in an organism of the *subtilis* group on the surface of agar.

On thick agar a film is produced by the union of such processes and their lateral projections. During its production many beautiful and fantastic designs may be seen.

Hutchinson (1906, p. 130) illustrates the growth during four hours of a thread-forming organism, *B. lactis albus*, apparently belonging to the slipping group. The observation ceased before general segmentation had occurred.

All the organisms belonging to the slipping group form films under suitable conditions. Under unsuitable conditions for film formation the larger and stronger members are apt to produce either colonies with radiating processes on moderately moist agar or rounded colonies on thin, dry agar. The smaller, weaker and slower growing members usually produce colonies which to the naked eye look more or less rounded. On closer inspection under the microscope, however, most of the types of colonies produced by the larger species can be recognised on a smaller scale. Exceptionally they produce colonies with long processes. *B. coli*, for example, may form a colony resembling that illustrated in Plate V, Fig. 11 (see Hutchinson (1906), Plate I, Fig. 5). All the organisms belonging to this group occasionally form long unsegmented filaments.

The mode of growth of the pathogenic anaerobic bacilli, the cocci and streptothrices has not yet been investigated, and attempts to follow the growth of *B. tuberculosis* were unsuccessful.

SUMMARY.

1. All the bacilli hitherto investigated, when growing on the surface or in the depth of agar, exhibit "post-fission" movements after division.

2. Four types of post-fission movement have been noticed, which may be termed "loop-forming," "folding," "snapping" and "slipping."

3. These types of post-fission movement seem to depend on the strength, adherence and mode of rupture of the capsule. In the "loop-forming" group the capsule is very strong and adherent and is very seldom ruptured. In the "folding" group the portion of the capsule uniting the adjacent bacilli in the chain is long and flexible. In the "snapping-group" the capsule seems to undergo partial rupture at the time of division, and in the "slipping" group it is completely ruptured.

4. "Loop-forming" post-fission movements are shown by *B. anthracis*, "folding" by *B. pestis* and certain organisms morphologically resembling *B. anthracis*, "snapping" by all diphtheroid organisms and "slipping" by organisms belonging to the typhoid-enteritidis-colon group, vibrios, the butter bacillus (Rabinowitch), *B. pyocyaneus*, *B. fluorescens*, *B. subtilis* and allied organisms and many non-pathogenic species.

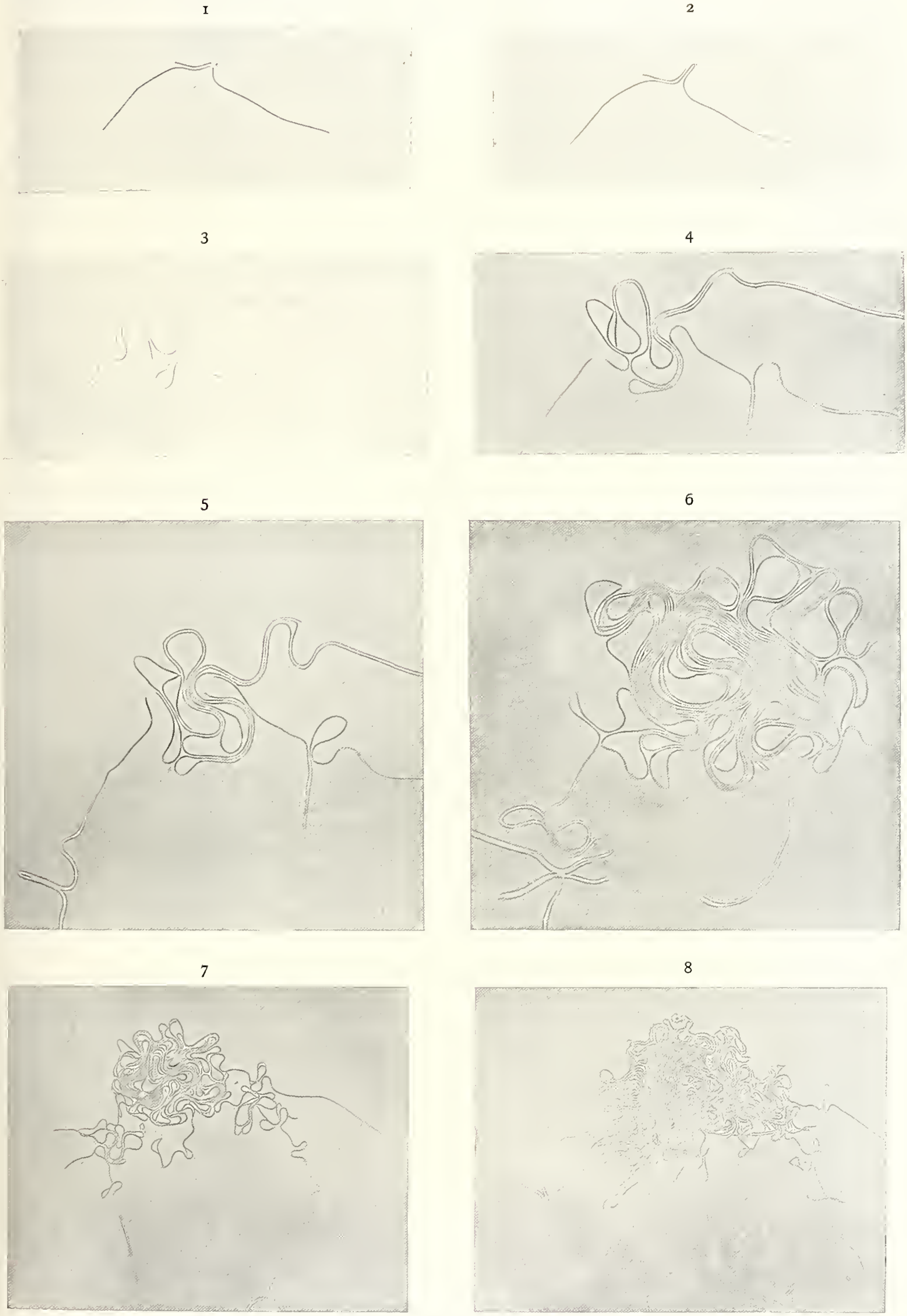

Plate I. The development of *B. anthracis* on the surface of agar.

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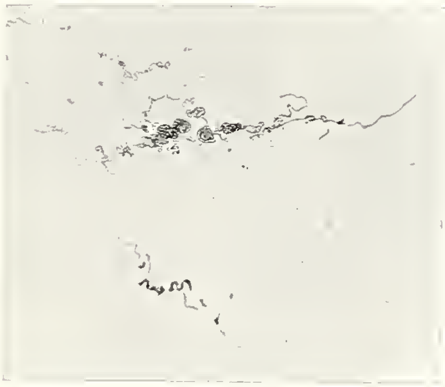

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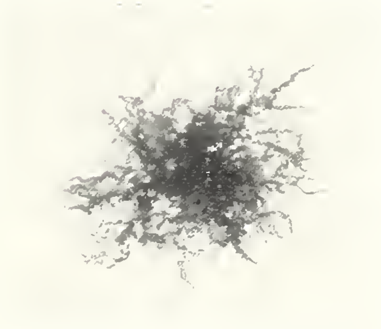

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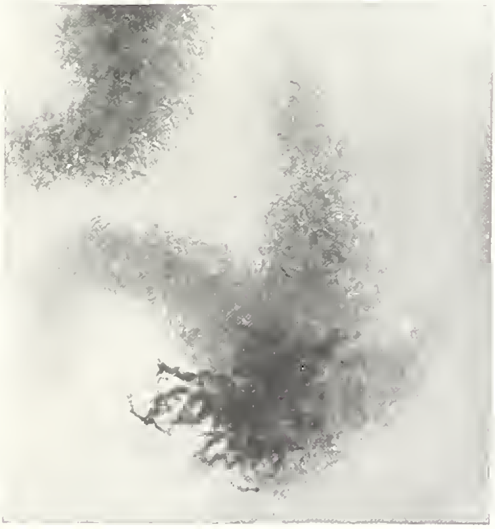

Plate II. The development of *B. anthracis* in the depth of agar.

BRITISH MUSEUM
Z D
NATURAL HISTORY

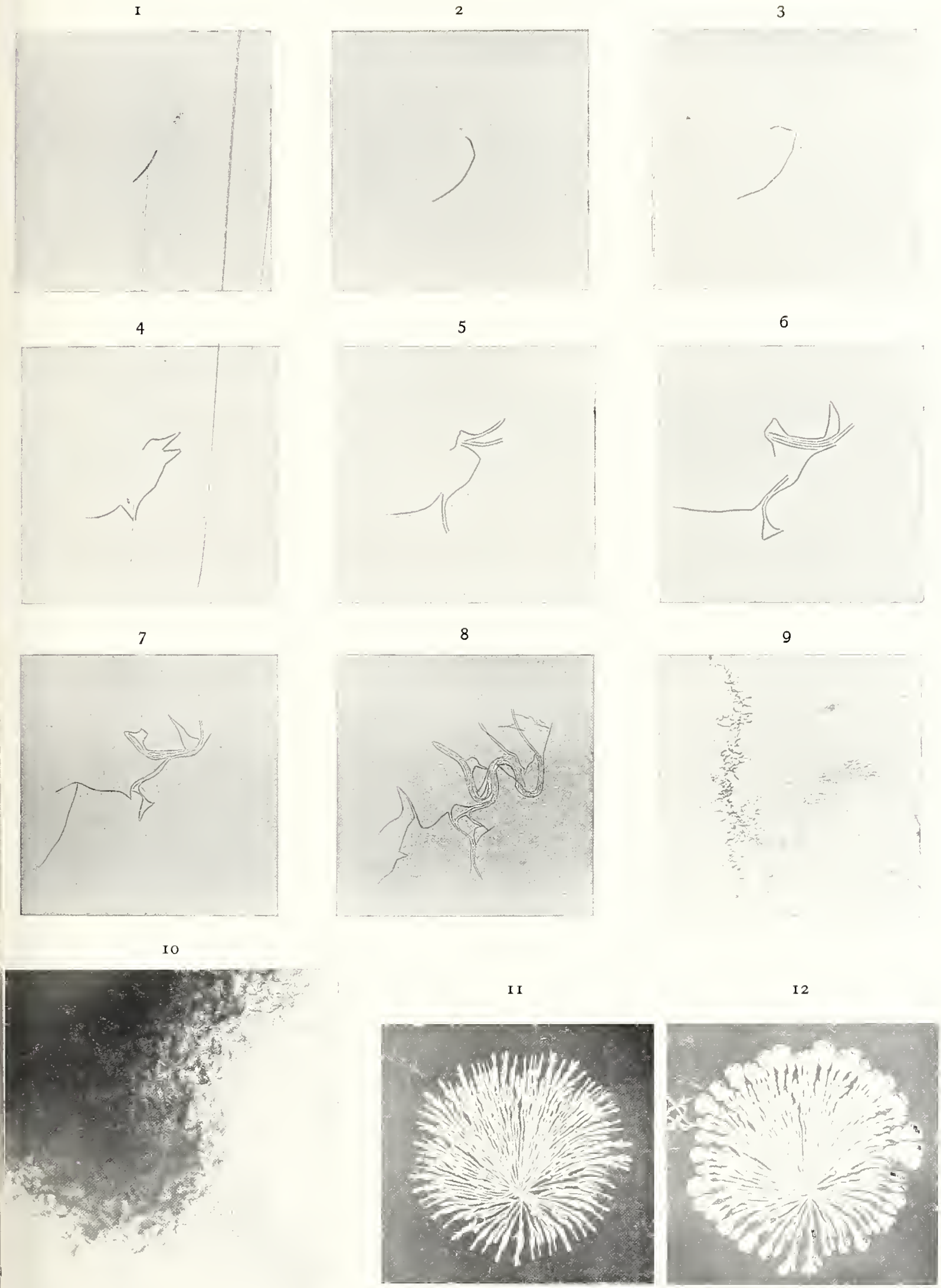

Plate III. The development of a "*folding*" organism on the surface of agar.

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NATURAL HISTORY

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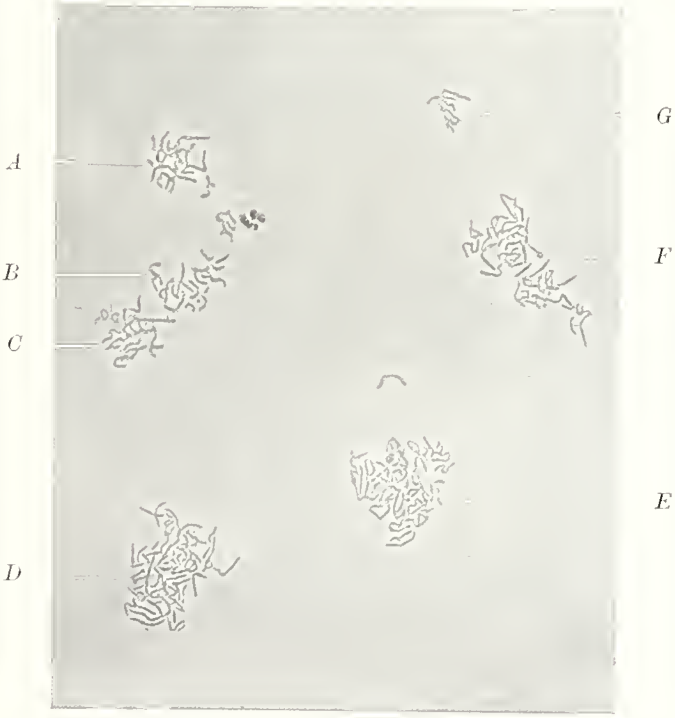

Plate IV. The development of *B. diphtheriae* on the surface of agar.

BRITISH MUSEUM
Z S D
NATURAL HISTORY

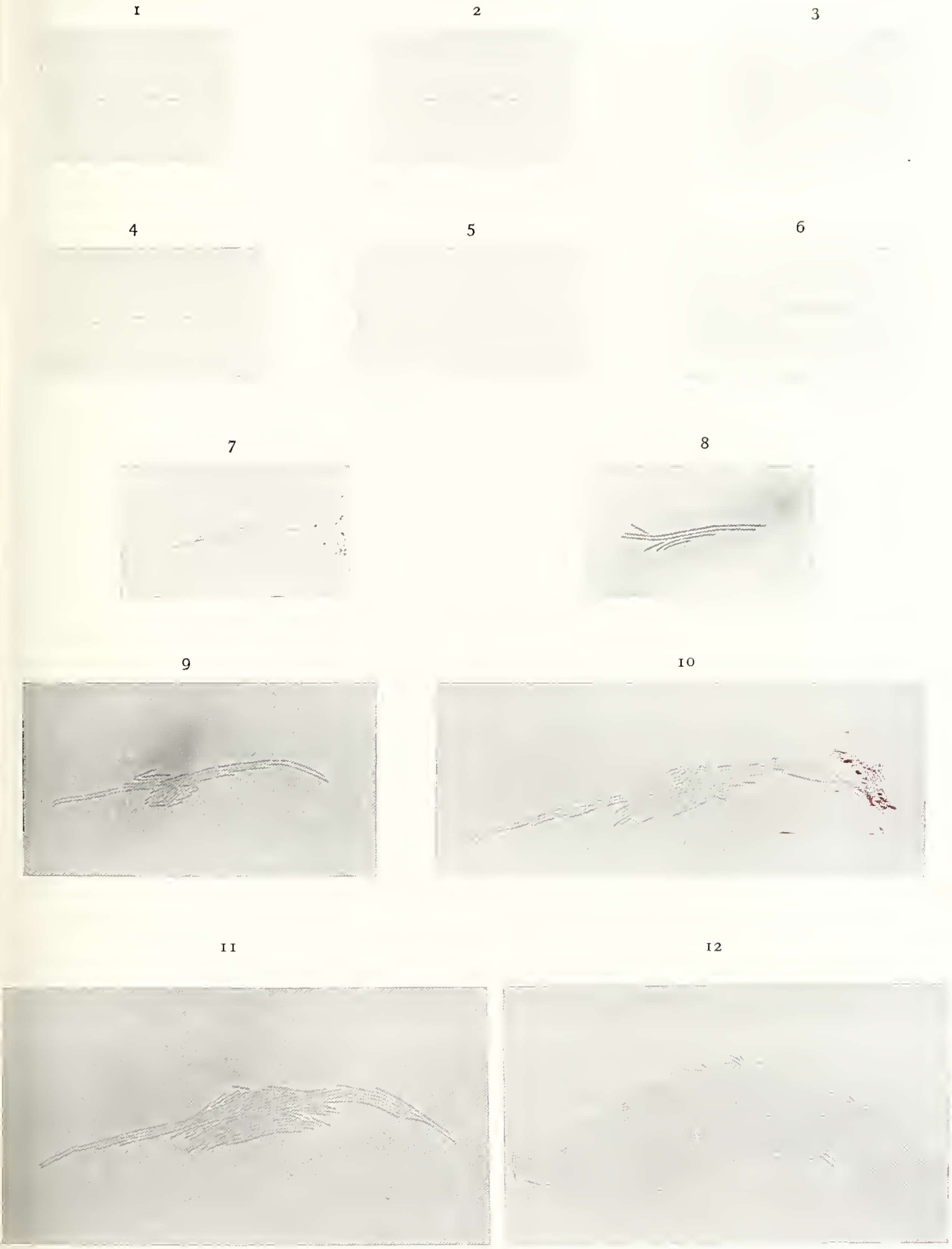

Plate V. The development of *B. subtilis* on the surface of agar.

BRITISH MUSEUM
Z S D
NATURAL HISTORY

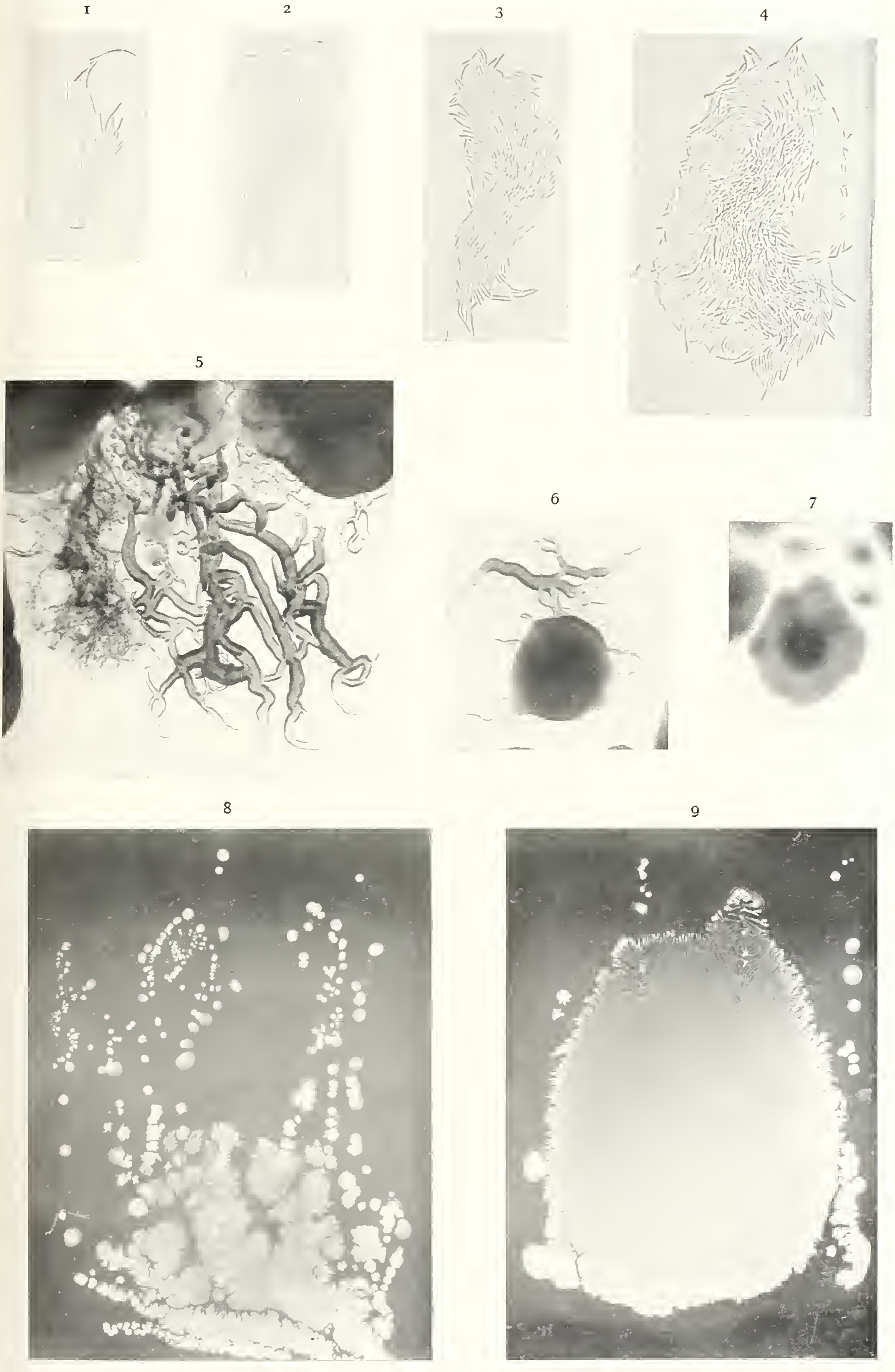

Plate VI. Surface colonies of *B. subtilis*.

BRITISH MUSEUM
Z D
NATURAL HISTORY

5. The characters of the colonies formed by organisms exhibiting loop-forming, folding and snapping post-fission movements are not markedly altered by the condition of medium under ordinary conditions of cultivation. When thickly sown on moist agar continuous growths are formed by all of them, but the edges of these growths resemble the edges of the separate colonies. All organisms exhibiting slipping post-fission movements form films on moist agar. Under unsuitable conditions for film formation the larger and stronger members produce either colonies with radiating processes on moderately moist agar, or rounded colonies on dry agar. The smaller, weaker, and slower growing members produce colonies which to the naked eye appear more or less rounded.

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SOME NOTES ON PARASITIC AND OTHER DISEASES OF FISH. 2ND SERIES.

By G. HAROLD DREW, B.A. Cantab.

Plate IX.

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Introduction.

Most of the specimens described in the following notes were collected by the International Fishery Commission's S.S. *Huxley*, in the North Sea, during the years 1905–09. They were preserved in dilute formalin in sea water, or in Bles's Fluid. Owing to the length of time during which the specimens had remained in the preservative, and in some cases owing to their large size, the fixation and preservation were not in every case as perfect as might have been wished, so that many of the finer histological details could not always be determined.

Only an account of the actual conditions found is given here. In the absence of opportunities for experimental work it is impossible to enter into the etiology or pathology of the diseases.

My thanks are due to the Marine Biological Association of the United Kingdom for their kindness in granting me a table at their Plymouth Laboratory.

Glugea shiplei, a new Sporozoon, parasitic in the muscles of Gadus luscus.

A small specimen of *Gadus luscus*, caught at Plymouth, was seen to have a number of oval translucent cysts irregularly scattered all over the muscles of the body. The fish only measured 10 centimetres in length, and was so small that on holding it up to the light, besides those cysts which were visible from their comparatively superficial position, a number of cysts could be seen deeply embedded in the muscles of the posterior part of the body.

The largest cysts averaged 5 mm. in length by 3 mm. in breadth, and contained a colourless gelatinous substance, in which one or more opaque white spots could be seen with the naked eye: the cyst wall was very thin and transparent. The cysts were present not only in the skeletal muscles, but also in those of the stomach and intestinal walls. Only muscular tissue was infected, and no trace of cysts could be found in any other tissue. The superficial cysts were not connected with the skin, and none had ruptured externally.

Some of the cysts were cut into, and the somewhat gelatinous contents turned out on a slide: under low powers of the microscope the jelly-like substance was seen to contain a number of circular bodies resembling nuclei, and the white opaque body appeared to consist of a dense mass of minute spores. On teasing out these spores, and examining under high powers, the spores were seen to be pyriform in shape, measuring $3{\cdot}5\mu$ long, by $2{\cdot}5\mu$ at their broadest axis (fig. 2). A small vacuole was present at the broader end, and a very minute body at the apex probably represents the polar capsule. In none of the spores could any filament be distinguished; attempts to cause protrusion of a filament by treatment with reagents were unsuccessful.

The shell of the spore was resistant to the action of strong sulphuric acid, and the dehiscence of the valves could not be determined. The vacuole was aniodinophilous. Portions of the muscular tissue of the trunk, containing these cysts, were excised and fixed in various fluids: somewhat unexpectedly those fixed in 5 % (of 30 %) formol alcohol gave the most satisfactory results. The tissue was embedded in paraffin and sectionised. Alum Haematëin, followed by Picro-Sauerfuchin, proved a satisfactory stain.

Sections showed the parasite encysted between the muscle fibres (figs. 1 and 3). The adventitious cyst wall was very slight, consisting in places of a thin layer of connective tissue with a few leucocytes, in other places it appeared to be entirely absent. The parasitic cyst wall consisted of an extremely thin structureless layer, closely attached to the adventitious cyst-membrane. Within the cyst the large amoeboid trophozoit could be seen: this had in all cases shrunk during the process of fixation, and no longer completely filled the cyst. It usually remained attached to the cyst wall in one or more places, and in some cases showed processes of the protoplasm running out towards the cyst wall, which at first sight resembled pseudopodia. I consider that these are only artificially produced by shrinking due to the fixation; they were not seen in every cyst, but occurred occasionally with every fixative employed.

The protoplasm of the trophozoit was slightly granular, and contained a large number of nuclei (fig. 3). Besides the normal nuclei, a number of pansporoblasts in various stages of spore formation were present. The devoloping pansporoblasts appear to migrate towards one or more centres in the protoplasm, with the result that one or more spherical masses of densely packed spores are formed, which are often visible to the naked eye as white opaque spots within the cysts. These masses of spores are surrounded by a condensation of the protoplasm, staining more strongly with Haematëin than the remainder, and containing a number of small, faintly staining, elongated nuclei with an ill defined, reticulate, arrangement of the chromatin. In the wall of this false membrane, small spherical masses consisting of a large number of spores could be seen, enclosed within their pansporoblast membranes, which, to use Gurley's (5) expression, are 'subpersistent as polysporophorous vesicles.' In the crowded central mass of spores the subpersistent pansporoblast membranes can be distinguished with a little difficulty, and can be seen to divide up the mass into divisions, each containing a large number of spores. In fig. 3 a slightly earlier stage of development is shown, in which a number of 'polysporophorous vesicles' are seen embedded in the central condensation of the protoplasm, which contains the faintly staining nuclei mentioned above.

In addition to the nuclei already described, there were a few large, elongated nuclei which possessed a well-marked central mass of chromatin, from which strands radiated towards the nuclear membrane.

These are strongly suggestive of the 'vegetative nuclei' described by Stempell (8) as occurring in *Nosema anomalum* (Monz). Unfortunately all the cysts examined were very nearly in the same stage of development, and so it was impossible to trace the history of these nuclei.

This sporozoon appears to be a previously undescribed species of *Glugea*. Adopting Minchin's (7) classification, its systematic position would be

Order	*Myxosporidia* (Bütschli)
Suborder	*Cryptocystes* (Gurley)
Family	*Glugeidae* (Thélohan)
Section	*Polysporogenea* (Doflein)
Genus	*Glugea* (Thélohan)
Species	*Shiplei* (spec. nov.)

Two cases of abdominal tumours occurring in Pleuronectes platessa.

Both the fish in which this condition was found were of large size, one measuring 63 centimetres, and the other 40 centimetres, in length. The larger of these was a female: the anterior half of the fish, with the viscera *in situ*, was preserved in dilute formalin in sea water. Of the smaller fish, only the liver and intestine, with the tumour attached, were sent to me, and no note as to the sex of the fish had been made. The condition in each appeared to be identical, both macro- and microscopically, so that only one case will be described in detail.

In the large female fish, there was an oval tumour in the body cavity, it was a soft red mass, the size of a hen's egg, situated posterior to the liver, and below the intestine, which it had displaced upwards. A thin transparent capsule was present, apparently formed of peritoneum, and the tumour was attached to the posterior surface of the liver by a thin pedicle consisting of the capsule alone. The growth was easily cut open, and was then seen to be homogeneous in character, but very soft and friable.

Small portions from various regions were embedded in paraffin and sectionised. The sections (fig. 4) showed glandular tissue consisting of cells of a columnar epithelial type. They were rather irregularly arranged, but circular acini could be distinguished. In many cases the lumina of the acini were partially filled with cells of an epithelial type, which had probably arisen by proliferation of the glandular cells forming the walls of the acini. Little fibrous interstitial tissue was present, but there were considerable areas apparently consisting of fat cells resembling the tissue found in lipomata. Isolated fat cells were also scattered irregularly about in the sections. The nucleus of each cell was situated in the broader basal part. Owing to the length of time during which the tissue had been preserved in formalin, the protoplasm often showed marked vacuolation, and it was difficult to make out cytological details. The whole tissue somewhat resembled that found in the mammalian pancreas, with the exception that there was nothing corresponding to the cell islets of Langerhans.

The liver was enlarged, and showed isolated nodules of a light yellow colour, slightly projecting from its surface. They were irregularly distributed over all the lobes, and in some cases measured almost half an inch in diameter. On cutting open the liver it was found that the nodules were also present in its deeper parts. Sections from different areas showed that the nodules were of the same nature as the large abdominal tumour, in fact histologically they were indistinguishable from it. In places the tissue of the nodules was not separated from that of the normal liver, but in other places there were indications of the presence of a thin capsule.

In the normal fish the pancreatic tissue is embedded in the liver, it consists of a mass of cells surrounding the branches of the portal vein: occasionally one or two small nodules of pancreatic tissue are found outside the liver in the mesentery. In these cases, tissue of a well-marked pancreatic type often surrounds small spherical masses of cells which are probably the homologues of the islets of Langerhans in the mammalian pancreas. In the liver the pancreatic tissue is often divided from the hepatic by a thin fibrous capsule, but it is possible to find places where there is no such division. The pancreatic tissue is not described as ever extending to the surface of the liver, except immediately round the point of entry of the portal vein, nor have I been able to find evidence of its presence in such localities in normal livers.

Thus it would seem that in these specimens there is an enormous hyperplasia of pancreatic tissue: the large oval tumour probably originated from a small mass of pancreatic tissue situated in the mesentery, and the nodules in the liver were possibly partly derived from the pancreatic tissue normally buried in the liver substance, though it is difficult to explain the presence of the superficial nodules which are found in places not normally occupied by pancreatic cells.

An explanation is to consider the condition an adeno-carcinoma of the pancreas, when it would be possible to account for the superficial nodules as secondary metastases, but this is scarcely justifiable considering the impossibility of determining cytological details in imperfectly preserved specimens. Dr Leo Loeb, of the Pathological Laboratory of the University of Pennsylvania, has kindly given me his opinion on sections of these tumours. He considers that the growth in places resembles a carcinoma and in others a lipoma. He also states that he has occasionally observed that gland-like tumours may produce appearances not unlike fat tissue.

The occurrence of Fibromata growing from the Opercula of Pleuronectes platessa.

This condition appears to be comparatively common. I have recently examined five cases in which hard fibromata had developed from the operculum, on either surface of the fish. The tumours were extremely hard and closely attached to the bone, in some cases they were partially ossified at the base. They consisted of very compact fibrous tissue, often showing a somewhat laminated structure. I have been unable to find anything that was suggestive of the cause of this condition.

A remarkable case of 'vitreous' degeneration in the muscles of Gadus aeglefinus, due to Sporozoon infection.

This specimen showed a slight swelling following the course of the lateral line of each side, but otherwise appeared normal on the surface. On cutting into the tissue in the neighbourhood of the lateral line, it was found that the swelling was due to the presence of a mass of brown, horny looking substance, which ran along on each side, embedded in the muscles immediately subjacent to the lateral line. This substance was hard, but cut readily and smoothly with the knife; sections were semitransparent, and appeared structureless under the microscope. The normal muscular tissue was not adherent to this horny mass, and showed no signs of inflammation even in its immediate neighbourhood: in fact shortly branching rods of this substance, measuring as much as $\frac{1}{2}$ inch in diameter, and one or two inches in length, could be easily withdrawn, clean and without any adherent tissue.

In places, white, pultaceous, masses of degenerated cells and fibrin were present, and on sectionising these masses, minute spores of some sporozoon, probably a species of *Glugea*, were made out. In the neighbourhood of these areas various stages of muscular degeneration were visible, and it was possible to trace the gradual disappearance of the muscular striae, until the whole muscle fibre had become changed to a structureless mass. The sarcolemma persists to a comparatively late stage, and then apparently vanishes, when the individual fibres fuse together into a structureless homogeneous mass. In places, the muscular fibres which had commenced to degenerate, were invaded by leucocytes (fig. 5), and in some cases the fibres were replaced by a mass of leucocytes and fibrin, though the sarcolemma was partially persistent A marked overgrowth of fibrous tissue between the fibres was commonly found.

This appears to be a remarkably advanced case of the 'dégénérescence vitreuse,' described by Thélohan (9) as occurring in the muscles of *Callionymus lyra* when infected by *Glugea destruans*. Unfortunately it was impossible to identify the sporozoon to which the degeneration was due, as only a few imperfectly preserved spores were present in the tissues, but from their shape and size, and the analogy of their effect on the host with that of *Glugea destruans*, it would appear probable that this is also a case of an infection by a species of *Glugea*.

A case of Epithelioma papulosum in Pleuronectes platessa.

This specimen (fig. 7) was a female, measuring 28 centimetres in length. The fish showed a number of circular, white, papillary elevations, some measuring as much as 14 mm. in diameter, irregularly distributed over the ocular surface.

Sections (fig. 6) cut at right angles to the surface of the skin, showed that the elevations consisted entirely of epidermal cells, superimposed so as to form a thick mass: they gradually thinned away at the periphery and became directly continuous with the normal epidermis. There was no invasion of the deeper tissues.

The condition appears to be similar to that found in the Carp disease (Pochenkrankheit) described by Hofer (6), and Doflein (2 and 3), who found that though the lesions of the skin were free from any parasitic infection, yet the kidney and sometimes the liver and testis or ovary, were infected with a *Myxobolus*, to which they gave the name of the *Myxobolus cyprini*. According to them, the skin lesions are an entirely secondary result, due to the formation of toxins by the organs infected with the sporozoon. On the other hand, Fiebiger (4) has found that apparently perfectly normal Carp are sometimes infected with the *Myxobolus cyprini*, and also has often been unable to detect the *Myxobolus* in fish obviously affected with Epithelioma papulosum.

Hofer (6) admits the lack of parallelism between the invasion of the kidney and the superficial lesions.

In the case of the plaice showing a superficial condition undoubtedly similar to the Epithelioma papulosum of the Carp, no trace of any sporozoon could be found, either in the lesions or in the tissues. A most careful examination was made, and portions of every organ were sectionised, but with absolutely negative results.

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DESCRIPTION OF PLATE IX.

Figs. 1, 2, and 3. To illustrate '*Glugea shiplei*, a new Sporozoon parasitic in the muscles of *Gadus luscus*.'

Fig. 4. To illustrate 'Two cases of abdominal tumours occurring in *Pleuronectes platessa*.'

Fig. 5. To illustrate 'A remarkable case of "Vitreous" degeneration in the muscles of *Gadus aeglefinus* due to Sporozoon infection.'

Figs. 6 and 7. To illustrate 'A case of Epithelioma papulosum in *Pleuronectes platessa*.'

Fig. 1. × 10. Section through a cyst, showing the trophozoit in which spore formation has begun, giving rise to a dense spherical mass of spores.

Semi-diagrammatic, the nuclei and spores are relatively too large.

Fig. 2. × 2,000. A single spore, showing vacuole and polar capsule.

Fig. 3. × 350. Section through the trophozoit, cyst wall and subjacent muscular tissue. The various nuclei of the trophozoit are shown, and the central condensation of the protoplasm containing pansporoblasts in an advanced state of development. Pansporoblasts in earlier stages of division are seen irregularly distributed in the protoplasm. The condition is a little less advanced than that shown in Fig. 1.

Fig. 4. × 350. Section through larger tumour, showing tissue resembling an adenocarcinoma above, and tissue resembling a lipoma below.

Fig. 5. × 350. Section across muscle fibres that have undergone complete 'vitreous' degeneration, many are invaded by leucocytes, and there is an overgrowth of fibrous tissue between the muscle fibres.

Fig. 6. × 350. Vertical section through one of the papules, showing great hyperplasia of epidermal cells which rest on the pigment layer of the dermis.

Fig. 7. × $\frac{1}{3}$. Showing distribution of papules on ocular surface of fish.

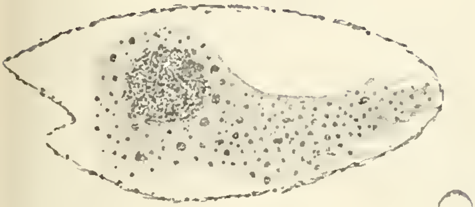

Fig. 1.

Fig. 2.

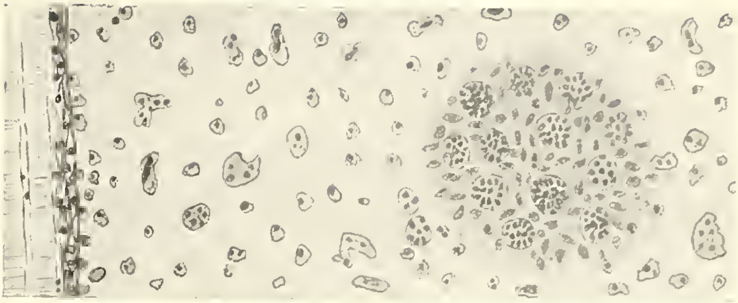

Fig. 3.

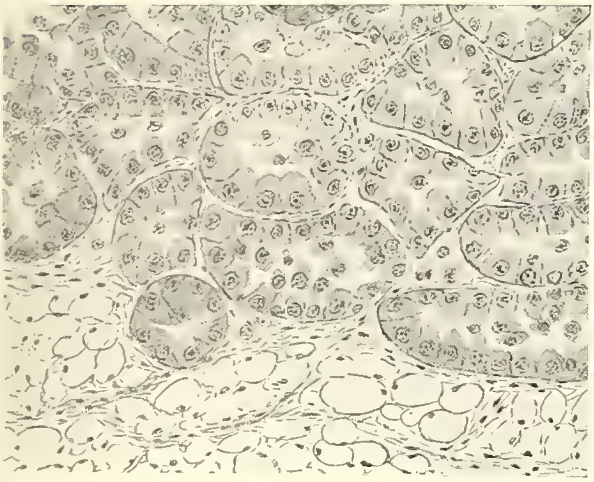

Fig. 4.

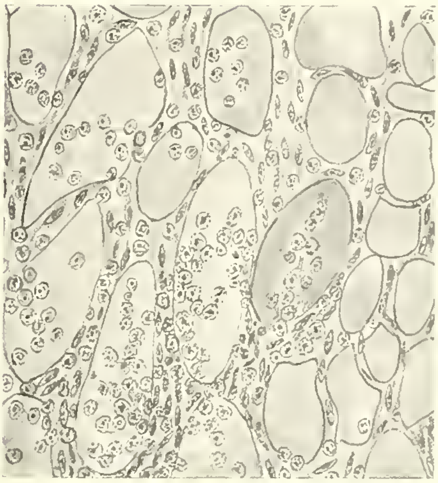

Fig. 5.

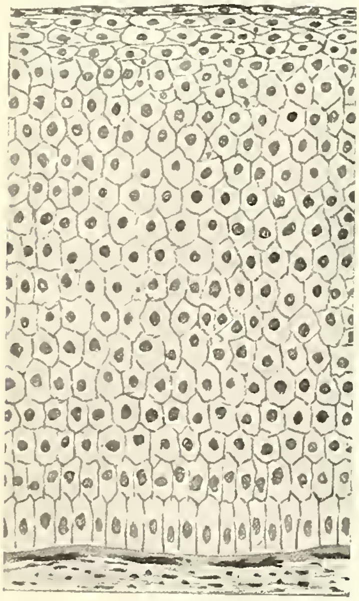

Fig. 6.

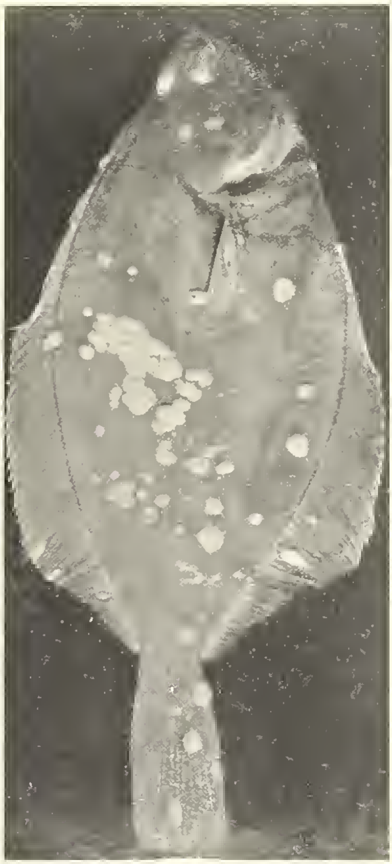

Fig. 7.

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SOME REMARKS ON THE GENUS LEUCOCYTOZOON.

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Protozoologist to the London School of Tropical Medicine.

NOTE. A reply to the criticisms contained in Dr Wenyon's paper will be published by Miss Porter in the next number of "Parasitology".

A GOOD deal of doubt still exists in many quarters as to the exact meaning of the term Leucocytozoon applied to certain Haematozoa. The term Leucocytozoaire was first used by Danilewsky in writing of certain parasites he had found in the blood of birds. In a later publication he uses the term Leucocytozoon for the same parasites though he does not employ it as a true generic title. In this latter sense it was first employed by Ziemann who named the parasite of an owl *Leucocytozoon danilewskyi*, thus establishing this parasite the type species of the new genus *Leucocytozoon*. It is perhaps hardly necessary to mention that Danilewsky and Ziemann both used this name because they considered the parasite in question to inhabit a leucocyte of the bird's blood. There has arisen some doubt as to the exact nature of this host-cell. Some authorities consider it to be a very much altered red blood corpuscle, some perhaps more correctly an immature red blood corpuscle, while others adhere to the original view of Danilewsky as to its leucocytic nature. It must be clearly borne in mind that the nature of the host-cell does not in any way affect the generic name *Leucocytozoon*. If it could be conclusively proved that the host-cell is in every case a red blood corpuscle the name *Leucocytozoon* would still remain as the generic title though it would have ceased to be descriptive.

Apparently it was a want of knowledge on this point that led James to apply the name *Leucocytozoon* to a totally distinct parasite which had been found by Bentley in India to attack certain leucocytes in the blood of dogs. In his paper describing this leucocytic parasite of dogs James discusses the various views as to the nature of the host-cell of the *Leucocytozoon* of birds and as he can find no agreement on this point he erroneously considers this sufficient ground for removing the name *Leucocytozoon* from the bird-parasite and applying it to the parasite of the dog which undoubtedly is within a leucocyte. Since the discovery by Bentley of the leucocytic parasite of dogs similar parasites have been described from the rat, mouse, cat, palm-squirrel and hare and for these the name *Leucocytozoon* has generally been employed, in spite of the fact that it has been constantly pointed out by Mesnil, Sambon and others that this name strictly belongs to the totally distinct parasite of birds.

Patton who has described several of these leucocytic parasites of mammals employs for them the generic name *Leucocytozoon*. Neither he nor James regards the leucocytic parasite of mammals as allied to the *Leucocytozoon* of birds. As James incorrectly justifies his use of the generic name *Leucocytozoon* for the dog-parasite by pointing out the doubt existing as to the true nature of the host-cell of the bird-parasite so Patton considers he is also justified in so doing, and he agrees with Laveran in considering the bird's parasites as belonging to the genus *Haemamoeba*, a genus in which Laveran includes the pigmented malarial parasites and their allies. It is evident that Patton thinks this would liberate the name *Leucocytozoon* from the bird-parasite. This is quite out of accord with the rules of nomenclature. Even if Laveran and Patton who follows him are correct in their view the name *Leucocytozoon* would remain as a synonym of the bird-parasite and could not be employed for any other form. As we shall see below the view that the *Leucocytozoon* of birds is really of the same genus as the pigmented parasites of malaria cannot be accepted.

Quite recently in *Science Progress* Miss Porter has reviewed our knowledge of the Leucocytozoa. She has attempted, as no one has done hitherto, to describe under this one name both the parasites of birds and the totally distinct parasite of mammals, with what success those who read the review will see. Such a grouping, it must be evident to anyone who has studied and compared both these parasites, can lead to nothing but confusion. Towards the close of her paper Miss Porter makes this statement "the name *Leucocytozoa* should be retained for *all* protozoal parasites of the leucocytes of vertebrates" in spite of the fact that the leucocytes of mammals are known to harbour several totally distinct parasites[1]. It is evident that Miss Porter has no knowledge of the *Leucocytozoon* of birds for her survey of the group is inaccurate and contradictory. With reference to the bird-parasites it is stated "Avian Leucocytozoa are said to exhibit sexual characters but this is somewhat doubtful" though earlier in the paper male and female forms are described. As a matter of fact the presence of both male and female forms in the peripheral circulation is one of the chief characteristics of the *Leucocytozoon* of birds. In this respect it agrees with the pigmented *Halteridium*. Again at the beginning of the paper Danilewsky's records are completely misinterpreted. In writing of the bird-parasites Danilewsky mentions having observed two forms of Polymitus in birds' blood, one of ordinary size and with grains of melanin, the other without grains of melanin but of very large dimensions. In a later publication he says he has found that the "leucocytozoaire" is only an intracellular stage in the development of the large Polymitus but he goes on to say that not all the "leucocytozoaires" develop into Polymitus. This is exactly what is known to occur and speaks for the accuracy of observation of this investigator. The *Leucocytozoon* of birds exhibits marked sexual characters. The male gametocyte gives rise to male gametes in much the same manner as the male forms of the malarial parasites while the female forms become rounded preparatory to fertilization by the male gamete. We thus find that Danilewsky was quite correct in his observation that only some of the "leucocytozoaire" develop into Polymitus. In view of these facts it is unfortunate that Miss Porter should state that "It is now known that the Leucocytozoa and the Polymitus of malaria have no connection." It is evident that Miss Porter has not read Danilewsky's papers with sufficient care.

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[1] The following parasites have been described from the leucocytes of mammals:—

1. Leishman-Donovan bodies.
2. The haemogregarines of dog, cat, rat, etc.
3. The *Toxoplasma gondii* of Nicolle and Marceaux.
4. A similar parasite described by A. Splendvie from the rabbit.
5. The curious trypanosome described by Cruz in Brazil.
6. The parasite of equine lymphangitis described by Doucloux.

For the sake of clearness I will give the characters of *Leucocytozoon*:—

1. They are exclusively parasites of birds for though Carini has described a *Leucocytozoon* from an amphibian it is exceedingly doubtful if this is a *Leucocytozoon* as it does not exhibit the sexual dimorphism which is such a distinctive characteristic of the avian parasites.

2. They are parasitic in cells which have been variously interpreted as leucocytes, red blood corpuscles, immature red blood corpuscles, etc.

3. They have the peculiar property of causing the host-cell to assume a characteristic spindle form[1]. The parasites themselves sur-

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[1] Miss Porter quotes me as admitting that sometimes the host-cell may have a spindle shape. If she will refer to my article she will see that I carefully state that there is practically always a spindle shape.

round the nucleus of host-cell which is found at the middle of the spindle.

4. Three forms of parasite can generally be recognised in the peripheral blood,—large deeply staining parasites which are the female gametocytes, pale staining parasites which are male gametocytes and lastly young forms which are possibly immature gametocytes.

5. The male gametocyte develops into a Polymitus or in other words it forms male gametes which are narrow whip-like organisms resembling the male gametes of the malarial parasites. These male gametes fertilize the female gamete and a motile zygote results.

6. The nucleus resembles that of *Halteridium*. The male nucleus consists of chromatin granules scattered about the centre of the parasite. The female nucleus is a more compact group of chromatin granules in the midst of which or to one side is a granule[1] definitely marked off from the rest by its larger size. It is possible that this granule is a karyosome.

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[1] There is a tendency for those who wish to establish the trypanosome nature of the intracorpuscular blood parasites, to describe any granule of chromatin which is larger than those of the main nuclear mass as homologous with the smaller chromatin body of trypanosomes. It must be remembered that extranuclear chromatin is of common occurrence in Protozoa so that it is exceedingly misleading to homologise any such granule of an intracorpuscular parasite with the definite micronucleus of trypanosomes. Woodcock has considered that his discovery of such a granule in *Halteridium* is the first definite evidence which has been brought forward in support of Schaudinn's contention that *Halteridium* has a trypanosome stage in its life history. It appears to me that Woodcock has failed to prove the one point necessary to support this hypothesis. It is not so much the discovery of such a granule that is required but the conclusive evidence that this granule is homologous with the micronucleus of a trypanosome. This evidence Woodcock has failed to produce. As a matter of fact similar granules had been described by several observers before Woodcock's discovery. In the Third Report of the Wellcome Research Laboratories, Khartoum, I have described this granule in the *Leucocytozoon* of the guinea-fowl and have figured it in the *Halteridium* on Plate XIII and again in the *Haemocystidium* on Plate XIV. It is equally undesirable to consider the similar granule in the piroplasmata as a micronucleus till it has definitely been proved to give rise to a flagellum or to have originally done so. In the case of the piroplasmata such a view has already produced considerable confusion with the Leishman-Donovan bodies. I quite agree with Calkins (Chap. IX, p. 269) who says in reference to the present tendency to group the Haemosporidia with the Mastigophora, "It is possible that future research will justify this step and that the large, relatively immobile blood parasites like Lankesterella of the frog, haemogregarines of turtles and tortoises, Karyolysus of lizards, haemoproteus of birds and plasmodium of man are like the Leishman-Donovan bodies only passing phases of some flagellated protozoon, but at the present time the evidence is not weighty enough to warrant such a step even as a working hypothesis. The weakness of the evidence, apparent as soon as reviewed may be briefly summarised as follows, etc.," and again on p. 270. "For these reasons, therefore, I believe it premature to separate the haemosporidia from the sporozoa."

7. The parasites are devoid of pigment, they are not enclosed in any cyst, they never leave the host-cell to move about in the blood plasma as do the haemogregarines, the asexual mode of reproduction is unknown nor is there any knowledge of the further development of the zygote and of the mode of transmission.

Such are the characters of the *Leucocytozoon* of birds. It will be evident to anyone who knows the group that its nearest relative is *Haemoproteus* (*Halteridium*) of the same host. It differs from *Halteridium* however in its large size, the absence of melanin pigment and the peculiar action it has upon its host-cell. It will be equally evident that it has no connection with the haemogregarines nor with the closely allied leucocytic parasites of mammals though Miss Porter would group the latter with the avian parasites.

The characters of these leucocytic parasites of mammals are as follows:—

1. They are parasitic in leucocytes of mammals.
2. They do not alter the shape of the host-cell to any extent though they may cause considerable change in its nucleus.
3. There is no sexual differentiation to be compared with that existing in the bird-parasites.
4. The nucleus is characteristic and resembles that of haemogregarines. It is quite unlike the nucleus of the *Leucocytozoon* and in staining reaction approaches that of the nucleus of the host-cell.
5. A cyst (cytocyst) is frequently though not invariably present. Within the cyst the parasite lies as a cylindrical body completely filling the cyst or as an elongate vermicule doubled upon itself in the shape of a **U**.
6. The parasites may leave their host-cell and their cyst and move about in the plasma as minute gregarines.
7. Asexual multiplication is by schizogony in a cell of some internal organ.
8. Sexual development has only been completely described in one case namely in the case of the leucocytic parasite of the rat. It has also been described but less completely for the similar parasite of dogs.

It is quite clear that these parasites are very closely allied to the haemogregarines and far removed from the *Leucocytozoon* of birds. Indeed if we look into these characters just enumerated were find there is not a single one of them which may not be applied to some typical haemogregarine. The fact that the host-cell is a leucocyte can hardly be regarded as a generic character and unless some marked difference in the life cycle of the leucocytic parasite of mammals and the haemogregarines of cold-blooded animals is discovered there is no reasonable course open but to include them in the same genus.

Miss Porter states that "The Leucocytozoa of mammals are very similar in general morphology to the Haemogregarina found in the red blood corpuscles of certain mammals" but she continues "surely the difference in habitat is sufficiently striking to separate the genera of strict mammalian Haemogregarines and Leucocytozoa." It is difficult to understand how mere habitat alone can in any way be converted into a generic character. In this, Miss Porter follows James and Patton who are stated to have the advantage, over Laveran and Mesnil, of first-hand knowledge of the group. Apparently Miss Porter imagines that Laveran and Mesnil and possibly others have not this first-hand knowledge, but I can assure her that in this she is mistaken.

In his paper on the leucocytic parasite of dogs James remarks "while it undoubtedly has affinities with the haemogregarines the fact that it is parasitic upon the leucocytes and that it represents a hitherto entirely unknown form of mammalian blood-infection would seem to justify its recognition as the type of a new genus," and Patton at the close of his description of the leucocytic parasite of the palm-squirrel makes this assertion, "It will be seen that the description of this parasite agrees in every detail with that of a haemogregarine." In spite of this complete agreement Patton regards the parasite as belonging to a distinct genus presumably because it is within a leucocyte. The question resolves itself into this—Can mere habitat be regarded as a generic character to separate one parasite from another when in other respects there is "agreement in every detail"?

It is very difficult to understand Patton's attitude towards his two *groups* Haemamoebina and Haemogregarina. In the genus *Haemamoeba* are included the malarial parasites of man the life cycles of which are so well known, the similar parasites of birds (*Proteosoma*), the *Halteridium* of birds which differs very much from the malarial parasites both in their asexual multiplication and mode of transmission and finally in this same genus he prefers to include the *Leucocytozoon* of birds though it displays such marked differences from the pigmented malarial parasites. A grouping together in a single genus of such diverse forms is a view accepted by very few authorities. If one compares such a parasite as the pigmented benign tertian parasite of malaria with a *Leucocytozoon* of a bird one can see at a glance that they are quite distinct.

The group *Leucocytozoon* of birds is a homogeneous one, the individual members of which show only slight variation so much so that it is exceedingly difficult to separate the species. There is no possibility of confusing any *Leucocytozoon* with the pigmented parasites with which Patton would group them so that the inclusion of such distinct forms in a single genus seems quite unnecessary. On the same line of argument one would be compelled to do away with all generic distinction in the Haemosporidia and to establish a single genus for the whole heterogeneous assemblage. It is certain that some of the Piroplasmata could be more easily confused with the malarial parasites than an avian *Leucocytozoon* should escape recognition. However it is to no purpose to pursue the argument further as Patton states that he prefers to consider the avian parasites as *Haemamoebae.* We find however that when Patton comes to consider the leucocytic parasite of the palm-squirrel though it "agrees in every detail with the haemogregarines" it is placed in a separate genus. I do not think even Patton would maintain that the *Leucocytozoon* of birds agrees in every detail with the parasites of malaria[1].

The most complete account of the development of a haemogregarine is that of the late Dr Miller of the parasite which he found in the leucocytes of rats. He seems to have been singularly fortunate in having at his disposal an unlimited amount of material. As a result of his researches he was able to follow the complete cycle of this parasite both in the rat and in the intermediate host—*Lelaps echidninus.* Now it occurred to him that the life cycle of this parasite of the rat would most probably be found to be very different from that of the type species of haemogregarine namely *Haemogregarina minima* of the frog, though the life cycle of the latter had not yet been discovered. The great difference in the two hosts seemed to warrant the conclusion that the life-cycles would be so distinct as to justify their inclusion in different genera. Accordingly Miller suggests for the rat-parasite the name *Hepatozoon perniciosum.* It must be very doubtful whether one is at liberty to create a new generic name on a mere probability for it must be remembered that the life cycle of the frog haemogregarine is not known. But the point I should like to emphasise is this that if the leucocytic parasites of mammals are to be separated from the haemogregarines the generic name will be *Hepatozoon.* We have seen that the generic name *Leucocytozoon* cannot be employed for the mammalian parasites as it is the true generic name of a distinct avian parasite. Towards the end of her paper Miss Porter says "However as the structure and life-history of avian leucocytozoa are still subjects of controversy and as the name *Leucocytozoon* was first applied to parasites of birds, and Lühe seems to restrict the name thereto, the generic name *Leucocytogregarina* might be used for the highly specialised parasites of mammalian leucocytes which have a different habitat from the strict Haemogregarines of red corpuscles." Miss Porter has still further confused the matter by introducing another name though it must be evident to her that if the leucocytic parasites of mammals require a distinct generic name the recently suggested name *Hepatozoon* of Miller will have priority.

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[1] In the case of the Leishman-Donovan bodies and the allied parasites, Patton has suggested that the generic name should be *Herpetomonas* and that the name *Leishmania* is to be abandoned. The evidence in support of this action is that certain *Herpetomonas* of the intestinal tract of non-biting arthropods exhibit stages in their development which are indistinguishable from the Leishman-Donovan bodies. There is no question of this *Herpetomonas* passing any part of its life-history in different hosts. The infection of an arthropod is by the accidental ingestion of cysts which have escaped from the intestine of an infected individual of the same species. In the case of the Leishman-Donovan body the condition is quite different. Part of its life-history is passed like a *Herpetomonas* in the intestine of some biting arthropod (bed-bug) but the other as a parasite within certain cells of a mammalian host. In the life-history of the Leishman-Donovan body there is then a definite alternation of hosts in one of which it exists as an intracellular parasite. This is a profound distinction which undoubtedly justifies its inclusion in a distinct genus. As the generic title of the Leishman-Donovan body and its allies the name *Leishmania* may well remain.

In an earlier paper in which Miss Porter describes a leucocytic parasite of the mouse she refers to recently described leucocytozoa and mentions one discovered by Sambon in the grouse. It was evident that here there was a misapprehension that the *Leucocytozoon* of birds was a parasite closely allied if not identical with the leucocytic parasites of mammals. As far as I can recall the literature this is the first instance in which such a mistake has been made. In her recent review Miss Porter has still further extended this mistake though the suggestion at the end of the review of a new generic name seems to indicate some doubt even in Miss Porter's mind as to the validity of this grouping.

All recent writers on the bird-parasites employ the true name *Leucocytozoon* and it is for these bird-parasites that this name must be used and not for the leucocytic parasite of mammals. As the complete life cycle of the haemogregarines has been followed in only one instance there are insufficient data for splitting up the group, so that it is at present safer to include in the group Haemogregarina the haemogregarines of the cold-blooded animals and the very similar parasites of the red blood corpuscles and leucocytes of mammals. If it be found that the life-history of the leucocytic parasites of mammals differs markedly from that of the type species of haemogregarine (*H. minima*) then the name *Hepatozoon* of Miller will have priority.

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THE TREATMENT OF SURRA IN HORSES BY MEANS OF ARSENIC AND ITS DERIVATIVES.

Thirty-two Cases of Successful Treatment.

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The value of arsenic in the treatment of trypanosomiasis is no new discovery.

Evans (1880), who described the first pathogenic trypanosome, recommended the administration of arsenic. Lingard (1899), for many years, investigated surra in India and treated twenty-one horses suffering from that disease with arsenious acid. One horse survived. His system of treatment was, the administration of arsenic in the form of Fowler's solution, commencing with 5 grains, twice daily and gradually increasing to 10 grains. Treatment extended over several months; the total amount of arsenic given, in some instances, amounted to over 2,000 grains. During the period of treatment intermissions of 30 to 50 days were recorded, but all the animals, with one exception, finally succumbed to the disease. Bruce (1897 and 1903), treating nagana in Zululand, gave 6 to 12 grains of arsenic in form of arsenite of soda to horses and asses suffering from that disease. Trypanosomes remained absent during the period of treatment, but reappeared immediately treatment ceased. Trélut (1907) claims to have cured cases of dourine by the daily administration of 3 to 6 grammes of arsenious acid. Archangeliky and Novikoff (1907) also record cases of dourine cured by them with arsenite of sodium and cacodylic acid. Marchal (1903) stated that he cured five stallions suffering from dourine by subcutaneous injections of 1 gramme of cacodylate of soda. Loeffler and Ruehs (1908) record a number of successful experiments on animals affected with nagana, which they treated with a solution of arsenious acid. They consider this solution to be a specific cure and a prophylactic for nagana. Laveran and Thiroux (1907–8) repeated these experiments and came to conclusions different from those of Loeffler and Ruehs.

At the present day the consensus of opinion regarding the value of the arsenic treatment in trypanosomiasis is in favour of the view that, though the drug exercises a marked influence on the course of most affections due to trypanosomes, it cannot be considered either a curative or a prophylactic agent.

In 1905, Thomas brought to notice the value of atoxyl, a derivative of arsenic, which contains 24 % of that drug and is said to be forty times less toxic than arsenious acid. It was supposed to owe its trypanocidal properties to the large amount of arsenic which could be administered without toxic effect in the form of atoxyl. The appearance of atoxyl has been followed by similar derivatives of arsenic, principally soamin, orsudan, kharsin prepared at the Messrs Burroughs and Wellcome Laboratory, and arsacetin and arsenophenylglycin, advocated by Professor Ehrlich (1908).

These arsanilates showed promise of the successful treatment of trypanosomiasis and gave a fresh stimulus to workers on the chemotherapy of trypanosomiasis.

Many remarkable results have been recorded with arsanilates used alone or in combination with arsenious acid, mercury and other drugs. In spite of the successes, which from time to time have been recorded both in clinical practice and laboratory experiments, following the use of these organic derivatives of arsenic in the treatment of trypanosomiasis, it is extremely doubtful whether any of these preparations can be regarded as a curative agent when used alone.

The most recent and what appears to be the most hopeful system of the chemo-therapy of trypanosomiasis is based on the combination of different trypanocides.

The combined method is said to have the advantage that the therapeutic value of each drug can be united against the parasites and a curative effect can be obtained by using doses much below those which are poisonous.

Among the most striking results of the combined therapy, Laveran and Thiroux (1908) cured guinea-pigs, which they had inoculated with surra, by using atoxyl and the trisulphide of arsenic. Thiroux and Teppaz (1909) treated horses, affected with Souma and Gambia horse-disease, with atoxyl and orpiment combined and with orpiment alone. Six months after the cessation of treatment the horses had not relapsed.

The same authors failed to cure horses suffering from surra by the atoxyl orpiment treatment.

Martin, Leboeuf and Ringenbach (1909) conclude from their experience of the treatment of human trypanosomiasis that the association of atoxyl and tartar emetic is the most effective of any which they have tried.

Broden and Rodhain (1909) agree that the results of the combined use of atoxyl and tartar emetic are much better than those obtained with either drug alone.

Moore, Breinl and Nierenstein (1907) assert the superiority of the association of atoxyl and a mercurial salt.

Loeffler and Ruehs (1908) advise the use of atoxyl with their arsenious acid solution.

Friedberger (1908) used atoxyl and thioglycollic acid with good results.

Mesnil, Nicolle and Aubert (1907) have tried atoxyl in association with a benzidine colour. Cures were obtained.

In the experiments which are dealt with in detail in another part of this paper, we have tested the value of atoxyl, arsenious acid, soamin, mercury and antimony salts alone, and in various combinations as curative agents in the treatment of surra in horses.

In the majority of the cases recorded, treatment was not commenced till the disease had become well established and at the period of the second paroxysm. The animals used for these experiments were worn out or lame pack ponies and a few aged army cast horses. Many of the ponies obtained for this purpose were in such poor condition that they succumbed to the disease in two to three weeks after inoculation and before treatment was commenced. The cast horses were in good condition and with them treatment has been most successful.

In all cases, where relapses occurred after treatment, the trypanosomes appeared in the circulation within a period of six weeks after the treatment had been discontinued. All the cases which are recorded in this paper have been under observation for periods varying from three to fourteen months after cessation of treatment. None have shown a relapse. All have been, therefore, classed as "recoveries."

The results of these experiments briefly summarised are as follows:

Atoxyl. The administration of atoxyl by the mouth failed to clear the circulation of parasites. After a subcutaneous injection trypanosomes disappeared from the blood in from six to eight hours. An intravenous injection acts more slowly than a subcutaneous one.

Continuous treatment with atoxyl for long periods failed to effect a cure. During the period of treatment trypanosomes remained absent from the circulation, but reappeared in every case shortly after treatment was discontinued.

Contrary to the experience of other workers, our experiments have indicated that arsacetin is equal in toxicity to atoxyl, and its administration has much the same effect on the trypanosomes.

In one case, pony no. C 30, where arsacetin was given in a single dose, which produced toxic symptoms, a cure has been effected.

Soamin. From the results of our experiments, I conclude that soamin is about equal in value to atoxyl. It is equally toxic when injected subcutaneously, but, administered by the mouth, it appears to be much less toxic than atoxyl.

In one case, pony no. C 31, where soamin was given in a single dose, which produced temporary paraplegia, the animal completely recovered and has shown no return of trypanosomes for eight and a half months.

Mercury. Mercury alone has little effect on the course of the disease. In combination with atoxyl the results were not so good as when atoxyl alone was used.

Antimony salts. Tartar emetic, in 1% solution, injected intravenously, has a rapid but temporary effect on the trypanosomes in the circulation.

Administered by the mouth, it has no influence on the course of the disease. Injected subcutaneously in very dilute solutions, it causes local irritation, frequently followed by necrosis of the skin, at the seat of inoculation.

The sodium antimonyl tartrate and lithium antimonyl tartrate gave no better results than the potassium salt, and were equally irritant when injected subcutaneously in horses and rats.

The combination of tartar emetic and mercury kept the circulation free from trypanosomes during the period of treatment only. A similar result followed the employment of tartar emitic and atoxyl, also of tartar emetic and arsenious acid solution. One pony, no. C 32, was cured by the treatment with tartar emetic, atoxyl and arsenious acid. The curative effect was probably due to the arsenious acid.

Arsenious acid. Ten cases, treated with arsenious acid alone, are recorded. In eight cases (C 1 to C 8) treatment has been successful. Five of these received ten doses, one eight, one five and one was cured by a single dose of arsenious acid. Two received four doses only and had a relapse. The arsenious acid was given in full sub-toxic doses at an interval of one day between each dose. The amount of arsenic was gradually increased as the animals showed a tolerance to the drug.

In addition to these cases here mentioned, a few animals, which were put under this treatment, showed an intolerance to the arsenic and each dose was followed by toxic symptoms. In these cases treatment was abandoned.

The administration of arsenious acid had no prophylactic effect even when the surra inoculation was made 24 hours after the dose of arsenic.

Arsenious acid and atoxyl. Two systems of combining these drugs were tried.

In the first, the atoxyl and arsenic were given alternately, five doses of each, with an interval of one day between each dose. In a former paper (Holmes, 1909) some of these cases described here were stated to have been treated with atoxyl and orpiment. In this same paper I drew attention to the fact that the sample of orpiment used was a commercial one, obtained from a dispensing chemist. On analysis this was found to contain 95 to 97°/₀ of free arsenious acid. Consequently, I have here included these cases as being treated with arsenious acid and atoxyl. In a number of experiments, some of which are not yet finished, we have tried treatment on the lines advised by Laveran and Thiroux with atoxyl and "orpiment precipité," but the results up to the present have been negative.

In the second system of treatment, the arsenious acid and atoxyl were given as follows. Arsenious acid solution (drench) (Loeffler and Ruehs), atoxyl (subcutaneously) and arsenious acid (bolus) were administered on consecutive days. These three doses were repeated once or twice after an interval of four days, and finally after an interval of eight days. The object sought in adopting this method of dosage was to bring the action of the arsenious acid solution and the atoxyl at the same time into the circulation, and also it was thought probable that, by administering arsenic at one time in form of drench and at another time in form of bolus, the gastric symptoms of arsenical poisoning would be less likely to occur and the drug would be better tolerated.

In a number of experiments we found that arsenious acid given in drench or bolus at a period of paroxysm freed the blood from trypanosomes in about 24 hours.

Atoxyl, subcutaneously, cleared the circulation in six to eight hours. Consequently, in these experiments, the atoxyl was injected 16 hours after the dose of arsenious acid.

Out of 16 cases treated on the first system seven had a relapse, nine were cured.

Sixteen ponies were treated on the second system, four had a relapse, 12 were cured.

CONCLUSIONS AND REMARKS.

(1) Exception may be taken to the practice of regarding as cured cases which have been under observation without relapse for a period of three to six months only. The fact that, among the animals treated, there has not been one case of relapse occurring after a period of six weeks from the cessation of treatment, and that the majority of cases quoted as cured have been under observation for three to 14 months, establishes reasonable grounds for including all these animals under the head of "recoveries."

(2) Arsenious acid is, undoubtedly, a specific for surra in horses. It must be administered in full sub-toxic doses and at intervals; and not in continuous daily dosage.

In the light of these results it is clear that, in the early experiments conducted by Lingard, Bruce and others, arsenic was given in too small an amount and in continuous treatment.

Animals cannot tolerate the requisite amount of arsenic if the dose be too frequently repeated. There is also the objection that by continuous small doses, not only does the animal body, but also the trypanosomes, acquire a certain amount of tolerance to arsenic.

(3) It is doubtful whether any advantage is gained by combining atoxyl with arsenious acid in the treatment of surra. The results of treatment with arsenious acid alone, given in 10 doses at an interval of one day, were superior to those where the arsenious acid was alternated with atoxyl. A single dose of arsenious acid alone keeps the blood free from trypanosomes for a period of 10 to 30 days and in some cases longer. When the same dose of arsenious acid is followed, 24 hours after, by an injection of a full amount of atoxyl, this period is not increased.

(4) Much has been written on the therapeutic action of atoxyl. It was at first supposed that atoxyl owed its trypanocidal properties to the fact that it contains a large proportion of arsenic and that it was simply a means of administering a large amount of that drug without producing toxic effect.

Levaditi and Yamanouchi (1908) consider that the curative power of atoxyl is due to its reduction and the formation of trypanotoxyl.

Ehrlich (1908) assumes that "the arsanilate as such exercises no action at all in the organism, but that only the very small portion which undergoes a reduction in the living organism is the actual lethal agent."

Some authors still believe that arsenic is split off in the body and that no action of atoxyl is due to this free arsenic.

Breinl and Nierenstein (1909) repeated the experiments of Levaditi and Yamanouchi, but only occasionally got positive results, and in these cases free arsenic was demonstrated in the solution of atoxyl. They conclude that "the assumption that a complicated reduction, product of atoxyl, is formed, is superfluous." According to their view the atoxyl action may be regarded as the result of a simple oxidation, by means of which arsenic is set free from the benzene ring. The finding of free arsenic in the urine, they say also, supports this view.

Tendron (1909), Wedemann (1908), Nierenstein (1908–09) and others found arsenic in the urine after the administration of atoxyl.

Uhlenhuth and Woithe (1908) are of opinion that the action of atoxyl is clearly not direct, but they repeated Levaditi and Yamanouchi's trypanotoxyl experiments with negative results. They do not agree with Ehrlich on his reduction theory. They think that the atoxyl stimulates the cells to a heightened production of substances which injure the parasites and that there is no direct action of reduced atoxyl.

All our observations on the action of atoxyl are in support of the view that a small amount of cleavage occurs and that the therapeutic effect is entirely due to the presence of free arsenic. We were unable to get any positive trypanotoxyl results in repeated experiments. Atoxyl is a very convenient method of administering arsenic hypodermically or intravenously, but, even in this form, it is impossible to give arsenic without toxic effect in quantities sufficient to permanently sterilise the tissues.

Both the toxic and therapeutic effects of arsenic and atoxyl administered intravenously and subcutaneously are much alike. Arsenic given hypodermically from 0·25 to 0·5 grammes, while it produces sloughing, frees the blood from trypanosomes at the same time and keeps the circulation free from the parasites for about the same period as 2 grammes of atoxyl injected subcutaneously.

When lethal doses are given subcutaneously or intravenously the symptoms of poisoning are produced about the third day. With atoxyl the kidneys are the seat of the chief macroscopical lesions, death, as a rule, being due to acute nephritis.

The same condition is observed when arsenic, in large doses, is administered subcutaneously or intravenously.

Both atoxyl and arsenic, when given in bolus, produce death more rapidly than when administered hypodermically. The lesions, in this case, are found in the stomach and intestines.

In several experiments, where atoxyl and arsenious acid were given at the same time, their toxic effects were undoubtedly combined. Ponies, about 250 lbs. weight, which could tolerate 1 gramme of arsenious acid and 50 c.c. of 4°/₀ atoxyl, when administered on separate days, showed symptoms of colic, dullness and loss of appetite when 0·5 gramme of arsenious acid was combined with the full dose of atoxyl.

(5) Our imperfect knowledge of the life cycle of the trypanosomes in the body is one of the difficulties in the problem of the treatment of the disease. The periodic appearance and disappearance of the trypanosomes in the circulation and the existence and location of the parasite during the intermission period are, as yet, insufficiently explained. The more generally accepted hypothesis, regarding the evolution of the trypanosomes in the body, appears to be that they multiply principally by longitudinal divisions, that a toxin, produced during the periods of paroxysm, is destructive to the majority of the mature parasites and that, during the period of intermission, the survivors multiply by division in the glands and organs.

In a paper, dealing with the evolution of the surra parasite (1904), I suggested that, after the breaking up of the parasites in the blood stream, the nucleus was capable of reforming into a new individual and depicted forms which appeared to be undergoing this process.

Breinl and Salvin Moore (1908) describe somewhat similar latent bodies which they found became encysted in the liver cells.

This hypothesis appears to be more in accordance with clinical observations and supplies a better explanation of the periods of paroxysm and intermission. At a paroxysm the parasites continue to increase in the blood for one, two or three days. If this increase was due to rapid division of the trypanosomes, one would expect this process to be most marked in the blood during the early part of this period. This is not the case. The time, when multiplication forms are most noticeable in the blood, is when the paroxysm is at its height and just preceding the downward curve. This, probably, indicates that the division process is an emergency method of multiplication called forth by products in the blood adverse to the life of the parasite. These new individuals, as well as the parent stock, become disintegrated to a certain extent and lose their flagellated form, but those destined to continue the species reform into a latent or cystic body which comes to rest in some internal organs, probably the liver or bone marrow, during the period of intermission, finally developing into flagellate forms, which again make their way into the blood streams.

The mature trypanosome is easily destroyed by arsenic, antimony and other drugs. The action of arsenic is direct and takes place when it is being carried through the tissues in the circulation. Arsenic acts but for a brief period, as it is rapidly eliminated from the circulation. The rapidity of the destructive action of arsenic on the trypanosomes is in direct proportion to the dose. Consequently, it is possible, if the dose is sufficiently large, to sterilise the tissues before the arsenic is removed. With smaller doses numbers of the parasites are not destroyed and, entering into a latent state, remain to cause a relapse. This hypothesis of the life stages of the trypanosomes would also explain the successful results of the treatment with arsenic given in full doses at intervals and the non-success of the continuous dosage with smaller amount. The toxic effect of arsenic on the system is cumulative, but the action on the trypanosomes is apparently not so. Arsenic in the circulation has, probably, little or no effect on the latent bodies, and the accumulated amount of the drug is stored up in the liver and other organs, where it is not brought in contact with the mature parasites.

I have to acknowledge the co-operation of Mr Cross, Assistant Bacteriologist, in these experiments, and the assistance of Dr Hartley, Physiological Chemist, in questions connected with chemistry.

The *Bulletin of the Bureau of Sleeping Sickness* has been extensively used for purposes of reference to other works on trypanosomiases.

TOXIC DOSE OF ARSENIOUS ACID, SOAMIN AND ATOXYL.

Arsenious acid.

The toxic dose for the horse is said to be (Law) 140 grains in solution or 14 grains in solid form; (Kaufmann) 3·4 grammes in solution or 45 grammes in solid form; Trélut administered 3 to 6 grammes daily in treatment for dourine.

In our experiments it was found that ponies of about 300 lbs. could tolerate repeated doses of 1 gramme without ill effect; that a dose of 2 to 3 grammes was fatal for ponies of this weight; that horses weighing from 900 to 1000 lbs. could not tolerate more that 3 grammes.

The arsenious acid was administered after feeding.

The toxic dose is 2 to 3 grammes for ponies, and 4 to 5 grammes for horses.

Arsenious acid administered in solution (Loeffler and Ruehs) has a more toxic effect than when given in bolus.

One gramme in solution is fatal for ponies weighing 250 lbs.

This is shown in the following table:

Pony no.	Weight lbs.	Dose of arsenious acid sol. c.c.	Results
1	225	600	No symptoms.
2	215	800	Colic, off feed for 2 days.
3	350	1000	Colic, off feed for 3 days.
4	250	1000	Died in 24 hours.

Arsenious acid injected subcutaneously.

The subcutaneous method of administering arsenious acid is not a suitable one. Even when highly diluted (1 in 1000) the injected fluid does not readily become absorbed and causes a large oedematous swelling, accompanied with pain and inflammation which, in some cases, results in necrosis of the skin.

Pony no. 5. 250 lbs., was injected with 100 c.c. arsenious acid (1 in 1000). Swelling at seat of inoculation. Pony was off feed for three days.

Mule no. 6. 427 lbs., was injected with 500 c.c. arsenious acid (1 in 1000); 250 c.c. was injected on either side. Large swellings at seat of inoculation resulted. The mule was off feed for two days. After several days the skin sloughed and the mule was destroyed.

Mule no. 7. 630 lbs., was injected with 0·5 gm. arsenious acid dissolved in 20 c.c. glycerine. Large swelling at seat of inoculation, which persisted for several days. Sloughing of the skin followed.

Pony no. 8. 220 lbs., received 0·75 gm. Arsenious acid dissolved in 50 c.c. of water containing 0·75 gm. sodium bicarbonate. Died in 16 hours.

Arsenious oxide injected intravenously.

Mule no. 9. 549 lbs., was injected with 100 c.c. of arsenious acid (1 in 1000). Showed no symptoms.

Mule no. 10. 550 lbs., was injected with 500 c.c. arsenious acid (1 in 1000). Mule was off feed for two days.

Horse no. 11. 900 lbs., was injected with 1 gm. arsenious acid dissolved in 250 c.c. of water containing 1 gm. of sodium bicarbonate. Symptoms of colic, followed after several days by paralysis of hind quarters. Destroyed.

Soamin.

Soamin administered by the mouth.

Pony no. 12 received at the rate of 6 gms. per 500 lbs.
,, 13 ,, ,, 10 ,, ,,
,, 14 ,, ,, 15 ,, ,,
,, 15 ,, ,, 20 ,, ,,
None showed any ill effect.

Soamin injected subcutaneously.

Pony no.	Weight lbs.	Dose gms.	Dose per 500 lbs. gms.	Results
16	200	2	5	No symptoms.
17	250	3	6	Colic, off feed 4 days.
18	250	4	8	Died.
19	500	10	10	Died.
20	250	5	10	Died.

The toxic dose is about 10 gms. per 500 lbs.

Atoxyl.

Atoxyl administered by the mouth.

Pony no. 21. Weight 229 lbs., dose 2·29 gms. (5 gms. per 500 lbs.). Slightly off feed for two days, no further symptoms.

Pony no. 22. Weight 300 lbs., dose 4·8 gms. (8 gms. per 500 lbs.). Off feed for three days, no further symptoms.

Pony no. 23. Weight 300 lbs., dose 6 gms. (10 gms. per 500 lbs.). Died in 17 hours.

The toxic dose is about 10 gms. per 500 lbs.

Atoxyl injected subcutaneously.

Pony no.	Weight lbs.	Actual dose gms.	Dose per 500 lbs. gms.	Results
24	670	6	4·47	Lived. No toxic effect.
25	420	5	5·95	,, ,,
26	330	4	6·06	,, ,,
27	390	5	6·41	,, ,,
28	460	7·5	8·15	,, ,,
29	250	5	10·0	Colic on 2nd day. Death on 5th day.
30	500	10	10·0	Lived. No toxic effect.
31	497	11·18	11·24	Death on 4th day.
32	220	5	11·36	,, ,,
33	405	10	12·34	Death with colic in 33 hours 40 minutes.
34	490	13·25	13·52	Death following day.
35	750	21·28	14·18	Death early following morn.

The toxic dose is about 10 gms. per 500 lbs.

Atoxyl injected intravenously.

Pony no.	Weight lbs.	Actual dose gms.	Dose per 500 lbs. gms.	Results
36	360	1·20	1·66	Lived, no toxic effect.
37	283	1·00	1·76	,, ,,
38	630	4·00	3·17	,, ,,
39	630	4·00	3·17	,, ,,
40	564	4·00	3·54	Lived, stood the dose on 4 consecutive days.
41	678	7·50	5·53	Lived.
42	574	7·50	6·53	Death.
43	565	7·50	6·63	,,
44	678	10·00	7·37	,,

The toxic dose is about 6 gms. per 500 lbs.

Having determined the approximate toxic dose of the various arsenic preparations used, the results of administering full sub-toxic doses were investigated.

Attention was directed to the following points.

(1) The effect on trypanosomes and determination of the period during which the blood remained free from trypanosomes after a single sub-toxic dose of each drug.

(2) The influence on the health and general condition of the animal.

(3) The comparison of the results produced by drugs administered by various channels (subcutaneously, intravenously and by the mouth).

(4) The effect of varying the interval between successive doses.

(5) The effect of combining various arsenic preparations, also of using arsenic in conjunction with other drugs.

The results are detailed in the data and tables following.

Treatment with atoxyl alone.

The value of a single dose of atoxyl injected subcutaneously. This has been estimated by the period during which the peripheral circulation remained free from trypanosomes. The atoxyl was administered at a period of paroxysm when the trypanosomes were numerous in the blood.

Pony no.	Weight lbs.	Dose		Period during which blood remained free from trypanosomes
45	250	30 c.c.	4 $^0/_0$ sol.	9 days.
46	660	60 ,,	12 ,,	5 ,,
47	300	150 ,,	2 ,,	6 ,,
47	300	200 ,,	2 ,,	7 ,,
47	300	100 ,,	2 ,,	4 ,,
48	450	100 ,,	2 ,,	6 ,,
48	450	200 ,,	2 ,,	7 ,,
49	500	100 ,,	2 ,,	3 ,,
49	500	150 ,,	2 ,,	5 ,,
49	500	200 ,,	2 ,,	8 ,,

A full dose of atoxyl, injected subcutaneously, freed the circulation from trypanosomes for 3 to 9 days.

Effect of atoxyl administered by the mouth.

The treatment was commenced when trypanosomes were numerous in the blood.

Pony no. 50. 300 lbs., received 4·8 gms. atoxyl in bolus (8 gms. per 500 lbs.). Pony was off feed for three days. No effect on trypanosomes. On seventh day received another 4 gms. atoxyl. No effect on trypanosomes. On ninth and eleventh day 4 gms. atoxyl were given, the number of trypanosomes in blood continued to increase. Treatment discontinued.

Pony no. 51. 229 lbs., treatment commenced on 5th day of disease. Received 2·29 gms. atoxyl in bolus (5 gms. per 500 lbs.). Pony was off feed for two days. No effect on trypanosomes.

Pony no. 52. 300 lbs., received 6 gms. atoxyl in bolus (10 gms. per 500 lbs.). Pony died in 17 hours.

Atoxyl administered by the mouth is about five times less toxic than arsenious oxide, but has no effect on the trypanosomes in the blood.

Effect of atoxyl injected subcutaneously.

A four per cent. solution of atoxyl was used except where otherwise stated.

Pony no. 53. 240 lbs. (intermittent dosage).

1st	day received	50 c.c.	9th	day received	40 c.c.
3rd	,, ,,	50 ,,	11th	,, ,,	40 ,,
5th	,, ,,	40 ,,	13th	,, ,,	40 ,,
7th	,, ,,	40 ,,			

Trypanosomes disappeared on 2nd day, but reappeared on 14th day.

Pony no. 54. 400 lbs. (intermittent dosage).

1st	day received	50 c.c.	7th	day received	50 c.c.
3rd	,, ,,	50 ,,	9th	,, ,,	50 ,,
5th	,, ,,	50 ,,			

Trypanosomes disappeared on 2nd day, but reappeared on 11th day.

Pony no. 55. 564 lbs. (continuous dosage).

1st	day received	50 c.c.	2	$^0/_0$ solution	8th	day received	100 c.c.	8	$^0/_0$ solution
2nd	,, ,,	50 ,,	2	,,	9th	,, ,,	100 ,,	8	,,
3rd	,, ,,	50 ,,	4	,,	10th	,, ,,	100 ,,	8	,,
4th	,, ,,	50 ,,	4	,,	11th	,, ,,	100 ,,	8	,,
5th	,, ,,	50 ,,	4	,,	12th	,, ,,	100 ,,	8	,,
6th	,, ,,	50 ,,	4	,,	13th	,, ,,	100 ,,	8	,,
7th	,, ,,	100 ,,	8	,,	14th	,, ,,	100 ,,	8	,,

Trypanosomes disappeared on the 2nd day, reappeared 30 days after the treatment was discontinued.

Pony no. 56. 630 lbs. (continuous dosage). Received eight doses of 50 c.c. of 4 % solution on consecutive days.

Trypanosomes disappeared after the first dose, but reappeared 13 days after the last dose.

Effect of atoxyl used intravenously.

Pony no. 57. 500 lbs. (continuous dosage). Received four injections of 50 c.c. of 2 % solution, followed by nine injections of 50 c.c. of 4 % solution on consecutive days.

Trypanosomes disappeared after first dose, but reappeared four days after last dose.

Pony no. 58. 560 lbs. (intermittent dosage). Received seven doses of 50 c.c. of 4 % solution on consecutive days. Interval of six days followed by four doses of 50 c.c. of 8 % solution on consecutive days.

Trypanosomes disappeared after first dose, but reappeared 29 days after the treatment was discontinued.

Atoxyl alone, administered in non-toxic doses, either by mouth, subcutaneously or intravenously, does not cure cases of surra.

Arsacetin.

(Sodium salt of acetyl—amino phenyl arsenic acid.)

This preparation is said to be much less toxic than the atoxyl.

Pony no. 59. 250 lbs., received subcutaneously 50 c.c. of 4 % solution. Died on 5th day.

Pony no. 60. 260 lbs., injected 100 c.c. of 4 % solution. Died on 6th day.

Pony no. 61. 450 lbs., injected 200 c.c. of 4 % solution. Died on 7th day.

Pony no. C 30. 310 lbs., injected 200 c.c. of 4 % solution. Off feed, colic, lying down, unable to rise. After nine days the symptoms passed off and the animal recovered. This pony was in an advanced stage of surra (16 days of the disease) and trypanosomes were numerous in the blood at the time of treatment. The trypanosomes were absent from the circulation next day. The animal has been kept under daily observation for 8½ months and shows no reappearance of the disease.

Arsacetin does not appear to be less toxic for the horse than atoxyl.

Treatment with arsenious acid alone.

Arsenious acid was administered in form of:

Arsenious acid (bolus).
Loeffler and Ruehs' solution (drench).

The effect of a single full dose administered at a period of paroxysm:

Arsenious acid (in bolus).

Pony no. 62. 300 lbs., dose 1 gm. The trypanosomes were absent from the circulation for 34 days.

Pony no. 63. 350 lbs., dose 1 gm. Trypanosomes disappeared from the circulation for 14 days.

Pony no. 64. 300 lbs., dose 1 gm. Trypanosomes disappeared from the circulation for 10 days.

Pony no. 65. 350 lbs., dose 1 gm. Trypanosomes disappeared for 23 days.

Mule no. 66. 500 lbs., dose 2 gms. Trypanosomes disappeared for 10 days.

Pony no. C 7. 300 lbs., dose 1 gm. No return of trypanosomes. Kept under observation 5¼ months.

Pony no. 68. 300 lbs., dose 1 gm. Trypanosomes disappeared for 21 days.

Pony no. 69. 300 lbs., dose 1 gm. Trypanosomes disappeared for 14 days.

Pony no. 70. 281 lbs., dose 1 gm. Trypanosomes disappeared from the circulation for 12 days.

Arsenious acid solution.

Pony no. 71. 225 lbs., received 800 c.c. Trypanosomes disappeared for 10 days.

Mule no. 72. 500 lbs., received 1200 c.c. Trypanosomes disappeared for five days.

Arsenious acid (continuous treatment).

Pony no. C 8. 350 lbs., 1st paroxysm. 14th day of disease.

1st day 1 gm. ars. ac.
3rd ,, 1 ,,
5th ,, 1·2 gms. ars. ac.
7th ,, 1·5 ,,
9th ,, 1·5 ,,
11th day 2 gms. ars. ac.
13th ,, 1 gm. ,,
15th ,, 1 ,,
17th ,, 1 ,,
19th ,, 1 ,,

No return of trypanosomes. Kept under observation 3¼ months.

Pony no. C 4. 350 lbs., 3rd paroxysm. 20th day of disease.

1st day 1 gm. ars. ac.
3rd ,, 1 ,,
5th ,, 1 ,,
7th day 1 gm. ars. ac.
9th ,, 1 ,,

Treatment stopped as pony was off feed and had an attack of colic. No return of trypanosomes. Kept under observation 3 months.

Pony no. C 6. 300 lbs., 3rd paroxysm. 19th day of disease.

1st day 1 gm. ars. ac.
3rd ,, 1 ,,
5th ,, 1 ,,
7th ,, 1 ,,
9th ,, 1 ,,
11th day 1·5 gms. ars. ac.
13th ,, 1·5 ,,
15th ,, 1·5 ,,
17th ,, 1·5 ,,
19th ,, 2 ,,

No reappearance of trypanosomes. Kept under observation 10½ months.

Pony no. C 3. 300 lbs., 4th paroxysm. 25th day of disease. This pony has been previously treated by another method which failed.

1st day	1 gm. ars. ac.	9th day	1 gm. ars. ac.
3rd ,,	1 ,,	11th ,,	1 ,,
5th ,,	1·2 gms. ars. ac.	13th ,,	1 ,,
7th ,,	1 gm. ,,	15th ,,	1 ,,

No reappearance of trypanosomes. Kept under observation 3½ months.

Horse no. C 1. 950 lbs., 3rd paroxysm. 17th day of disease.

1st day	1 gm. ars. ac.	9th day	2·5 gms. ars. ac.
3rd ,,	1·5 gms. ars. ac.	11th ,,	2·5 ,,
5th ,,	1·5 ,,	13th ,,	3 ,,
7th ,,	2 ,,		

After this last dose of 3 gms. the horse was off his feed for three days and treatment was suspended for 10 days.

24th day 2 gms. ars. ac. 26th day 2 gms. ars. ac.
28th day 2 gms. ars. ac.

No reappearance of trypanosomes. Kept under observation 6 months.

Horse no. C 2. 900 lbs., 3rd paroxysm. 19th day of disease.

1st day	1 gm. ars. ac.	9th day	2·5 gms. ars. ac.
3rd ,,	1·5 gms. ars. ac.	11th ,,	2·5 ,,
5th ,,	1·5 ,,	13th ,,	3 ,,
7th ,,	2 ,,		

After the last dose of 3 gms. the horse was off feed for some days and treatment was suspended for 10 days.

24th day 2 gms. ars. ac. 26th day 2 gms. ars. ac.
28th day 2 gms. ars. ac.

No reappearance of trypanosomes. Kept under observation 6 months.

Pony no. C 5. 250 lbs., 1st paroxysm. 6th day of disease.

1st day	1 gm. ars. ac.	11th day	1·5 gms. ars. ac.
3rd ,,	1 ,,	13th ,,	1·25 ,,
5th ,,	1 ,,	15th ,,	1·25 ,,
7th ,,	1 ,,	17th ,,	1·25 ,,
9th ,,	1·25 gms. ars. ac.	19th ,,	1·5 ,,

No reappearance of trypanosomes. Kept under observation 8 months.

Pony no. C 7. 300 lbs., 1st paroxysm. 6th day of disease.

1st day 1 gm. ars. ac.

Pony was off feed for several days and no further treatment was given.

No return of trypanosomes. Kept under observation 5¼ months.

Pony no. 78. 250 lbs., 2nd paroxysm. 15th day of disease. Four doses of arsenious acid were given at intervals of four days.

1st day 1 gm. ars. ac.	11th day 1 gm. ars. ac.
6th ,, 1 ,,	16th ,, 1 ,,

Trypanosomes reappeared 18 days after last dose.

Pony no. 79. 300 lbs., 2nd paroxysm. 12th day of disease. Four doses of arsenious acid were given at intervals of one day.

1st day 1 gm. ars. ac.	5th day 1 gm. ars. ac.
3rd ,, 1 ,,	7th ,, 1 ,,

Trypanosomes reappeared 21 days after last dose.

Preventive treatment.

Pony no. 80. 400 lbs.

1st day 1 gm. ars. ac.
4th ,, 1 ,,

After four days' interval was inoculated with surra. Trypanosomes appeared eight days after the inoculation.

Pony no. 81. 280 lbs.

1st day 50 c.c. of 4 % atoxyl.
3rd ,, 1·5 gms. ars. ac.

After seven days' interval was inoculated with surra. Trypanosomes appeared six days after the inoculation.

Pony no. 82. 270 lbs.

1st day 1 gm. ars. ac.
6th ,, 0·5 gm. ars. ac.
7th ,, inoculated with surra.
10th ,, 0·5 gm. ars. ac.
15th ,, inoculated with surra.

Trypanosomes appeared on 17th day.

Pony no. 83. 230 lbs. Treated as Pony no. 81.

Trypanosomes appeared 9th day.

Pony no. 84. 300 lbs. Treated as Pony no. 82.

Trypanosomes appeared 12th day.

Mule no. 85. 450 lbs. 1st day received intravenously 100 c.c. arsenious acid in solution (1 in 1000). 5th day was inoculated with surra.

11th day trypanosomes appeared in the circulation.

Mule no. 86. 500 lbs. 1st day 3 gms. arsenious acid dissolved in glycerine. 4th day was inoculated with surra.

12th day trypanosomes appeared in the circulation.

Orpiment.

In these experiments chemically pure arsenious sulphide was used.

Pony no. 73. 250 lbs., received 6 gms. No effect on trypanosomes.

Pony no. 74. 230 lbs., received 7 gms. Trypanosomes disappeared for 10 days.

Pony no. 75. 307 lbs., received 8 gms. Trypanosomes disappeared for five days.

Pony no. 76. 416 lbs., received 10 gms. Trypanosomes disappeared for eight days.

Pony no. 77. 523 lbs., received 10 gms. Trypanosomes disappeared for seven days.

Treatment with atoxyl and arsenic.

Treatment was commenced at the first or second period of paroxysm, when trypanosomes were numerous in the circulation. Freshly prepared four per cent. solutions of atoxyl were used subcutaneously. Arsenious acid was administered in bolus.

Several of the earlier experiments in which the animals died from over dosage are not here recorded.

The first seven cases detailed are experiments in which the treatment did not meet with success; the following nine are cases where the result has been successful.

The atoxyl and arsenic were administered alternately, leaving one day interval between each dose.

Pony no. 87. 300 lbs., 2nd paroxysm. 16th day of disease. (Intermittent dosage.)

1st day 50 c.c. atoxyl.
3rd ,, 0·5 gm. ars. ac.
5th ,, 50 c.c. atoxyl.
7th ,, 0·5 gm. ars. ac.
9th ,, 50 c.c. atoxyl.
11th day 0·5 gm. ars. ac.
13th ,, 50 c.c. atoxyl.
15th ,, 0·5 gm. ars. ac.
17th ,, 50 c.c. atoxyl.
19th ,, 0·5 gm. ars. ac.

The trypanosomes disappeared after the first dose. Reappeared 4 days after the treatment ceased. The dose of ars. ac. was insufficient.

Pony no. 88. 600 lbs., 1st paroxysm. 4th day of disease. (Intermittent dosage.)

1st day 50 c.c. atoxyl.
3rd ,, 2 gms. ars. ac.
5th ,, 75 c.c. atoxyl.
7th ,, 3 gms. ars. ac.
9th ,, 100 c.c. atoxyl.
11th day 2 gms. ars. ac.
13th ,, 100 c.c. atoxyl.
15th ,, 2 gms. ars. ac.
17th ,, 100 c.c. atoxyl.
19th ,, 2 gms. ars. ac.

Trypanosomes disappeared from the circulation after first dose, but reappeared 7 days after last dose.

Pony no. 89. 500 lbs., 2nd paroxysm. 18th day of disease. This pony had received previous treatment which failed. (Intermittent dosage.)

1st day	70 c.c. atoxyl.	11th day	1 gm. ars. ac.
3rd ,,	2 gms. ars. ac.	13th ,,	100 c.c. atoxyl.
5th ,,	100 c.c. atoxyl.	15th ,,	1 gm. ars. ac.
7th ,,	1 gm. ars. ac.	17th ,,	100 c.c. atoxyl.
9th ,,	100 c.c. atoxyl.	19th ,,	1 gm. ars. ac.

Trypanosomes disappeared after the first dose, but reappeared 8 days after the last dose.

Pony no. 90. 560 lbs. (Intermittent dosage.)

1st day	50 c.c. atoxyl.	11th day	2 gms. ars. ac.
3rd ,,	0·5 gm. ars. ac.	13th ,,	50 c.c. atoxyl.
5th ,,	50 c.c. atoxyl.	15th ,,	2 gms. ars. ac.
7th ,,	2 gms. ars. ac.	17th ,,	50 c.c. atoxyl.
9th ,,	75 c.c. atoxyl.	19th ,,	2 gms. ars. ac.

Trypanosomes disappeared after the first dose, but reappeared 28 days after the last dose.

Pony no. 91. 280 lbs., 1st paroxysm. 6th day of disease. (Intermittent dosage.)

1st day	50 c.c. atoxyl.	7th day	1 gm. ars. ac.
3rd ,,	1 gm. ars. ac.	9th ,,	50 c.c. atoxyl.
5th ,,	50 c.c. atoxyl.	11th ,,	1·5 gms. ars. ac.

Trypanosomes disappeared after the first dose, but reappeared 21 days after the last dose.

Pony no. 92. 500 lbs., 1st paroxysm. 4th day of disease. (Simultaneous dosage.)

1st day	40 c.c. atoxyl	and	2 gms. ars. ac.
2nd ,,	40 ,,	,,	2 ,,
3rd ,,	40 ,,	,,	2 ,,

Trypanosomes disappeared on 2nd day, but reappeared 9 days after the last dose.

Pony no. 93. 300 lbs., 1st paroxysm. 5th day of disease. (Simultaneous dosage.)

1st day	20 c.c. atoxyl	and	1 gm. ars. ac.
2nd ,,	20 ,,	,,	1 ,,
3rd ,,	20 ,,	,,	1 ,,
4th ,,	20 ,,	,,	1 ,,

Trypanosomes disappeared after first dose, but reappeared 12 days after last dose.

In the above seven cases of failure, pony no. 87 did not receive sufficiently large doses of ars. ac., a fact which was recognised by the result of latter experiments. Ponies nos. 89 and 80 had been previously treated with atoxyl alone and the trypanosomes had acquired a resistance to this drug. Pony no. 91 received six doses only, which were not sufficient. Ponies nos. 92 and 93 were treated with atoxyl and ars. ac., administered at the same time. This system did not meet with any success.

In the following cases treatment has apparently effected a cure.

Pony no. C 11. 250 lbs., 5th day after inoculation. (Intermittent dosage.)

1st day	50 c.c. atoxyl.	11th day	0·5 gm. ars. ac.
3rd ,,	0·5 gm. ars. ac.	13th ,,	50 c.c. atoxyl.
5th ,,	50 c.c. atoxyl.	15th ,,	0·5 gm. ars. ac.
7th ,,	0·5 gm. ars. ac.	17th ,,	50 c.c. atoxyl.
9th ,,	50 c.c. atoxyl.	19th ,,	0·5 gm. ars. ac.

No return of trypanosomes. Kept under observation 4 months.

Horse no. C 17. About 900 lbs., spontaneous case. In an advanced stage of the disease when treatment was commenced. (Intermittent dosage.)

1st day	100 c.c. atoxyl.	11th day	1·5 gms. ars. ac.
3rd ,,	1·5 gms. ars. ac.	13th ,,	100 c.c. atoxyl.
5th ,,	100 c.c. atoxyl.	15th ,,	2 gms. ars. ac.
7th ,,	1·5 gms. ars. ac.	17th ,,	100 c.c. atoxyl.
9th ,,	100 c.c. atoxyl.	19th ,,	2 gms. ars. ac.

Trypanosomes reappeared 11 days after the last dose. A second treatment with increased doses was given. (Intermittent dosage.)

1st day	125 c.c. atoxyl.	11th day	3 gms. ars. ac.
3rd ,,	3 gms. ars. ac.	13th ,,	150 c.c. atoxyl.
5th ,,	150 c.c. atoxyl.	15th ,,	3 gms. ars. ac.
7th ,,	3 gms. ars. ac.	17th ,,	150 c.c. atoxyl.
9th ,,	150 c.c. atoxyl.	19th ,,	3 gms. ars. ac.

No return of trypanosomes. Under observation 12 months.

Pony no. C 15. 190 lbs., 1st paroxysm. 3rd day of disease. (Intermittent dosage.)

1st day	30 c.c. atoxyl.	11th day	1·5 gms. ars. ac.
3rd ,,	1 gm. ars. ac.	13th ,,	30 c.c. atoxyl.
5th ,,	30 c.c. atoxyl.	15th ,,	1·5 gms. ars. ac.
7th ,,	1 gm. ars. ac.	17th ,,	30 c.c. atoxyl.
9th ,,	30 c.c. atoxyl.	19th ,,	1·5 gms. ars. ac.

No return of trypanosomes. Kept under observation 12 months.

Horse no. C 16. 280 lbs., 1st paroxysm. 1st day of disease. (Intermittent dosage.)

1st ,,	50 c.c. atoxyl.	10th day	50 c.c. atoxyl.
2nd ,,	60 ,,	12th ,,	2 gms. ars. ac.
4th ,,	1·5 gms. ars. ac.	14th ,,	50 c.c. atoxyl.
6th ,,	50 c.c. atoxyl.	16th ,,	2 gms. ars. ac.
8th ,,	1·5 gms. ars. ac.	18th ,,	50 c.c. atoxyl.

No return of trypanosomes. Kept under observation 12 months.

Pony no. C 15. 220 lbs., 1st paroxysm. 5th day of disease. (Intermittent dosage.)

1st day 50 c.c. atoxyl.
3rd ,, 1 gm. ars. ac.
5th ,, 50 c.c. atoxyl.
7th ,, 1 gm. ars. ac.
9th ,, 50 c.c. atoxyl.
11th day 1 gm. ars. ac.
13th ,, 50 c.c. atoxyl.
15th ,, 1 gm. ars. ac.
17th ,, 50 c.c. atoxyl.
19th ,, 1 gm. ars. ac.

No return of trypanosomes. Kept under observation 13 months.

Pony no. C 12. 300 lbs., 1st paroxysm. 4th day of disease. (Intermittent dosage.)

1st day 50 c.c. atoxyl.
3rd ,, 1 gm. ars. ac.
5th ,, 50 c.c. atoxyl.
7th ,, 1 gm. ars. ac.
9th ,, 50 c.c. atoxyl.
11th day 1 gm. ars. ac.
13th ,, 50 c.c. atoxyl.
15th ,, 1 gm. ars. ac.
17th ,, 50 c.c. atoxyl.
19th ,, 1 gm. ars. ac.

No return of trypanosomes. Kept under observation 10 months.

Pony no. C 14. 250 lbs., 1st paroxysm. 1st day of disease. (Intermittent dosage.)

1st day 50 c.c. atoxyl.
3rd ,, 0·5 gm. ars. ac.
5th ,, 50 c.c. atoxyl.
7th ,, 0·5 gm. ars. ac.
9th ,, 50 c.c. atoxyl.
11th day 0·5 gm. ars. ac.
13th ,, 50 c.c atoxyl.
15th ,, 50 ,,
17th ,, 0·7 gm. atoxyl.

No return of trypanosomes. Kept under observation 14 months.

Mule no. C 9. 300 lbs., 2nd paroxysm. 13th day of disease. (Intermittent dosage.)

1st day 50 c.c. atoxyl.
3rd ,, 0·5 gm. ars. ac.
5th ,, 50 c.c. atoxyl.
7th ,, 1 gm. ars. ac.
9th ,, 50 c.c. atoxyl.
11th day 1·25 gms. ars. ac.
13th ,, 50 c.c. atoxyl.
15th ,, 1·25 gms. ars. ac.
17th ,, 50 c.c. atoxyl.
19th ,, 1·5 gms. ars. ac.

No return of trypanosomes. Kept under observation 7½ months.

Mule no. C 10. 500 lbs., 2nd paroxysm. 12th day of disease. (Intermittent dosage.)

1st day 50 c.c. atoxyl.
3rd ,, 1·5 gms. ars. ac.
5th ,, 50 c.c. atoxyl.
7th ,, 1·5 gms. ars. ac.
9th ,, 60 c.c. atoxyl.
11th day 2 gms. ars. ac.
13th ,, 60 c.c. atoxyl.
15th ,, 2 gms. ars. ac.
17th ,, 60 c.c. atoxyl.
19th ,, 2 gms. ars. ac.

No return of trypanosomes. Kept under observation 7½ months.

In the following 16 experiments the atoxyl and arsenious acid were administered on a different system.

A dose of arsenious acid in solution (Loeffler and Ruehs) in drench was administered at a period of paroxysm, this was followed on consecutive days by a dose of atoxyl subcutaneously and a dose of arsenious acid in bolus. These three doses were repeated once or twice at intervals of four days and once after an interval of eight days. In a few instances the intervals between the doses varied.

In some cases a dose of atoxyl subcutaneously was given first, which cleared the circulation of trypanosomes within 24 hours.

This system of treatment has been the most successful of any tried. Out of 16 animals treated by this method, 12 were cured. In four cases the treatment failed : in three instances probably from insufficient doses, and in one case the interval between the doses was increased to six and 12 days.

All the animals stood the treatment well and rapidly improved in condition.

Pony no. C 18. 300 lbs., 1st paroxysm. 4th day of disease.

1st day 40 c.c. atoxyl.
3rd ,, 300 c.c. ars. ac.
4th ,, 30 c.c. atoxyl.
5th ,, 1 gm. ars. ac.
Four days interval.
10th day 300 c.c. ars. ac.
11th day 30 c.c. atoxyl.
12th ,, 1 gm. ars. ac.
Eight days interval.
21st day 300 c.c. ars. ac.
22nd ,, 30 c.c. atoxyl.
23rd ,, 1 gm. ars. ac.

Trypanosomes appeared in the circulation four days after the last dose. A second course of treatment with increased doses was given.

1st day 400 c.c. ars. ac.
2nd ,, 50 c.c. atoxyl.
3rd ,, 1 gm. ars. ac.
Two days interval.
6th day 400 c.c. ars. ac.
7th ,, 50 c.c. atoxyl.
8th ,, 1 gm. ars. ac.
Four days interval.
13th day 400 c.c. ars. ac.
14th ,, 50 c.c. atoxyl.
15th ,, 1·5 gms. ars. ac.
Eight days interval.
24th day 400 c.c. ars. ac.
25th ,, 60 c.c. atoxyl.
26th ,, 2 gms. ars. ac.

No return of trypanosomes. Kept under observation 7½ months.

Pony no. C 23. 300 lbs., 2nd paroxysm. 13th day of disease.

1st day 500 c.c. ars. ac.
2nd ,, 50 c.c. atoxyl.
3rd ,, 1 gm. ars. ac.
Four days interval.
8th day 500 c.c. ars. ac.
9th ,, 50 c.c. atoxyl.
10th ,, 1 gm. ars. ac.
Four days interval.
15th day 500 c.c. ars. ac.
16th ,, 50 c.c. atoxyl.
17th ,, 1 gm. ars. ac.
Eight days interval.
26th day 500 c.c. ars. ac.
27th ,, 50 c.c. atoxyl.
28th ,, 1 gm. ars. ac.

No return of trypanosomes. Kept under observation 9 months.

Pony no. C 24. 300 lbs., 2nd paroxysm. 13th day of disease.

1st day 500 c.c. ars. ac.
2nd ,, 50 c.c. atoxyl.
3rd ,, 1 gm. ars. ac.
Four days interval.
8th day 500 c.c. ars. ac.
9th ,, 50 c.c. atoxyl.

10th day 1 gm. ars. ac.
Four days interval.
15th ,, 400 c.c. ars. ac.
16th ,, 50 c.c. atoxyl.
17th ,, 1 gm. ars. ac.
Eight days interval.
26th day 400 c.c. ars. ac.
27th ,, 50 c.c. atoxyl.
28th ,, 1 gm. ars. ac.

No return of trypanosomes. Kept under observation 9 months.

Pony no. C 20. 300 lbs., 4th paroxysm. 20th day of disease. This pony had been treated by another method without success.

1st day 600 c.c. ars. ac. sol.
2nd ,, 40 c.c. atoxyl.
3rd ,, 2 gms. ars. ac.
Four days interval.
8th day 600 c.c. ars. ac. sol.
9th ,, 40 c.c. atoxyl.
15th day 2 gms. ars. ac.
Eight days interval.
24th ,, 400 c.c. ars. ac. sol.
25th ,, 40 c.c. atoxyl.
27th ,, 1 gm. ars. ac.

No return of trypanosomes. Kept under observation 9½ months.

Pony no. C 29. 370 lbs., 1st paroxysm. 2nd day of disease.

1st day 50 c.c. atoxyl.
2nd ,, 400 c.c. ars. ac. sol.
3rd ,, 50 c.c. atoxyl.
4th ,, 2 gms. ars. ac.
Four days interval.
9th day 60 c.c. ars. ac. sol.
10th ,, 50 c.c. atoxyl.
11th ,, 2 gms. ars. ac.

No further treatment was given as the animal was off feed and showed toxic symptoms. No return of trypanosomes. Kept under observation 11 months.

Pony no. C 28. 215 lbs., 2nd paroxysm. 13th day of disease.

1st day 400 c.c. ars. ac. sol.
2nd ,, 50 c.c. atoxyl.
3rd ,, 1 gm. ars. ac.
Four days interval.
8th day 300 c.c. ars. ac. sol.
9th ,, 40 c.c. atoxyl.
10th ,, 0·5 gm. ars. ac.
Four days interval.
15th day 300 c.c. ars. ac. sol.
16th ,, 40 c.c. atoxyl.
17th ,, 0·75 gm. ars. ac.
Eight days interval.
26th day 300 c.c. ars. ac. sol.
27th ,, 40 c.c. atoxyl.
28th ,, 0·75 gm. ars. ac.

No return of trypanosomes. Kept under observation 8½ months.

Pony no. C 25. 260 lbs., 2nd paroxysm. 13th day of disease.

1st day 400 c.c. ars. ac. sol.
2nd ,, 50 c.c. atoxyl.
3rd ,, 1 gm. ars. ac.
Four days interval.
8th day 400 c.c. ars. ac. sol.
9th ,, 50 c.c. atoxyl.
10th ,, 1 gm. ars. ac.
Four days interval.
15th day 300 c.c. ars. ac. sol.
16th ,, 40 c.c. atoxyl.
17th ,, 0·75 gm. ars. ac.
Eight days interval.
26th day 300 c.c. ars. ac. sol.
27th ,, 40 c.c. atoxyl.
28th ,, 0·75 gm. ars. ac.

No return of trypanosomes. Kept under observation 9 months.

Pony no. C 26. 260 lbs., 2nd paroxysm. 13th day of disease.

1st day 500 c.c. ars. ac. sol.
2nd ,, 50 c.c. atoxyl.
3rd ,, 1 gm. ars. ac.
Four days interval.
8th day 500 c.c. ars. ac. sol.
9th ,, 50 c.c. atoxyl.
10th ,, 1 gm. ars. ac.
Four days interval.
15th day 400 c.c. ars. ac. sol.
16th ,, 50 c.c. atoxyl.
17th ,, 1 gm. ars. ac.
Eight days interval.
26th day 400 c.c. ars. ac. sol.
27th ,, 50 c.c. atoxyl.
28th ,, 1 gm. ars. ac.

No return of trypanosomes. Kept under observation 9 months.

Pony no. C 21. 245 lbs., 1st paroxysm. 5th day of disease.

1st day 50 c.c. atoxyl.
3rd ,, 300 c.c. ars. ac. sol.
4th ,, 30 c.c. atoxyl.
5th ,, 1 gm. ars. ac.
Four days interval.
10th day 300 c.c. ars. ac. sol.
11th day 30 c.c. atoxyl.
12th ,, 1 gm. ars. ac.
Eight days interval.
21st day 300 c.c. ars. ac. sol.
22nd ,, 30 c.c. atoxyl.
23rd ,, 1 gm. ars. ac.

No return of trypanosomes. Kept under observation 10 months.

Pony no. C 22. 280 lbs., 2nd paroxysm. 8th day of disease.

1st day 500 c.c. ars. ac. sol.
2nd ,, 50 c.c. atoxyl.
3rd ,, 1 gm. ars. ac.
Two days interval.
6th day 450 c.c. ars. ac. sol.
7th ,, 50 c.c. atoxyl.
8th ,, 1 gm. ars. ac.
Two days interval.
11th day 450 c.c. ars. ac. sol.
12th ,, 50 c.c. atoxyl.
13th day 1 gm. ars. ac.
Four days interval.
18th day 450 c.c. ars. ac. sol.
19th ,, 50 c.c. atoxyl.
20th ,, 1 gm. ars. ac.
Eight days interval.
29th day 400 c.c. ars. ac. sol.
30th ,, 50 c.c. atoxyl.
31st ,, 1 gm. ars. ac.

No return of trypanosomes. Kept under observation 8 months.

Pony no. C 19. 240 lbs., 3rd paroxysm. 18th day of disease.

1st day 300 c.c. ars. ac. sol.
2nd ,, 50 c.c. atoxyl.
3rd ,, 1 gm. ars. ac.
Six days interval.
10th day 300 c.c. ars. ac. sol.
11th ,, 50 c.c. atoxyl.
12th ,, 1 gm. ars. ac.

Trypanosomes appeared 9 days after last dose.

A second course of treatment was given.

1st day 400 c.c. ars. ac. sol.
2nd ,, 50 c.c. atoxyl.
3rd ,, 1 gm. ars. ac.
Three days interval.
7th day 400 c.c. ars. ac. sol.
8th ,, 50 c.c. atoxyl.
9th day 1 gm. ars. ac.
Four days interval.
14th day 400 c.c. ars. ac. sol.
15th ,, 50 c.c. atoxyl.
16th ,, 1 gm. ars. ac.

No return of trypanosomes. Kept under observation 9 months.

Pony no. C 27. 270 lbs., 2nd paroxysm. 13th day of disease.

1st day 50 c.c. atoxyl.
3rd ,, 600 c.c. ars. ac. sol.
4th ,, 1 gm. ars. ac.
6th ,, 50 c.c. atoxyl.
8th ,, 500 c.c. ars. ac. sol.
9th day 1 gm. ars. ac.
11th ,, 50 c.c. atoxyl.
13th ,, 500 c.c. ars. ac. sol.
14th ,, 1 gm. ars. ac.

This pony was treated on a different system. The arsenious acid solution and arsenious acid were given on consecutive days and the atoxyl alternately, after one day interval.

No return of trypanosomes. Kept under observation 9 months.

Three other ponies, treated in the same method, showed a return of the disease a few days after treatment ceased.

Pony no. 94. 300 lbs., 2nd paroxysm. 13th day of disease.

1st day 300 c.c. ars. ac. sol.
2nd ,, 50 c.c. atoxyl.
3rd ,, 1 gm. ars. ac.
Four days interval.
8th day 300 c.c. ars. ac. sol.
9th ,, 50 c.c. atoxyl.
10th day 1 gm. ars. ac.
Eight days interval.
19th day 300 c.c. ars. ac. sol.
20th ,, 50 c.c. atoxyl.
21st ,, 1 gm. ars. ac.

Trypanosomes reappeared 30 days after last dose.

Pony no. 95. 300 lbs., 1st paroxysm. 4th day of disease.

1st day 300 c.c. ars. ac. sol.
2nd ,, 25 c.c. atoxyl.
3rd ,, 1 gm. ars. ac.
Four days interval.
8th day 300 c.c. ars. ac. sol.
9th ,, 25 c.c. atoxyl.
10th day 1 gm. ars. ac.
Eight days interval.
19th ,, 300 c.c. ars. ac. sol.
20th ,, 25 c.c. atoxyl.
21st ,, 1 gm. ars. ac.

Trypanosomes reappeared 14 days after last dose.

Pony no. 96. 230 lbs., 1st paroxysm. 4th day of disease.

1st day 300 c.c. ars. ac. sol.
2nd ,, 30 c.c. atoxyl.
3rd ,, 1 gm. ars. ac.
Four days interval.
8th day 300 c.c. ars. ac. sol.
9th ,, 30 c.c. atoxyl.
10th day 1 gm. ars. ac.
Eight days interval.
19th day 300 c.c. ars. ac. sol.
20th ,, 30 c.c. atoxyl.
21st ,, 1 gm. ars. ac.

Trypanosomes reappeared 7 weeks after last dose.

Pony no. 97. 320 lbs., 3rd paroxysm. 21st day of disease. This pony had received other treatment without success.

1st day 50 c.c. atoxyl.
4th ,, 300 c.c. ars. ac. sol.
5th ,, 50 c.c. atoxyl.
6th ,, 1 gm. ars. ac.
Six days interval.
13th day 300 c.c. ars. ac. sol.
14th day 50 c.c. atoxyl.
15th ,, 1 gm. ars. ac.
Twelve days interval.
28th day 400 c.c. ars. ac. sol.
29th ,, 50 c.c. atoxyl.
30th ,, 1 gm. ars. ac.

Trypanosomes reappeared 12 days after last dose.

TREATMENT WITH SOAMIN.

Effect of a single dose.

Pony no. 98. 350 lbs., 1st paroxysm. 2nd day of disease. 60 c.c. of 4 $^0/_0$ soamin subcutaneously. Trypanosomes disappeared from the circulation for nine days.

Pony no. 99. 407 lbs., 1st paroxysm. 3rd day of disease. 60 c.c. of 4 $^0/_0$ soamin subcutaneously. Trypanosomes disappeared from the circulation for six days.

Mule no. 100. 500 lbs., 1st paroxysm. 2nd day of disease. 100 c.c. of 4 $^0/_0$ soamin subcutaneously. Trypanosomes disappeared from the circulation for 12 days.

Pony no. 101. 250 lbs., 1st paroxysm. 4th day of disease. 100 c.c. of 4 $^0/_0$ soamin subcutaneously. Pony died the following day.

Pony no. C 31. 250 lbs., 3rd paroxysm. 22nd day of disease. 100 c.c. of 10 $^0/_0$ soamin subcutaneously. The pony was off feed and unable to rise for several days after which he gradually recovered. No reappearance of trypanosomes. Kept under observation 8½ months.

Pony no. 102. 298 lbs., 2nd paroxysm. 12th day of disease.

1st day 50 c.c. of 4 $^0/_0$ soamin.
3rd ,, ,, ,,
5th ,, ,, ,,
7th ,, ,, ,,

Pony was off feed and treatment was discontinued. Trypanosomes reappeared 14 days after last dose.

Soamin administered by the mouth in large doses (20 gms. per 500 lbs.) had no effect on the trypanosomes in the blood. Continuous treatment by the same method had no influence on the course of the disease.

TREATMENT WITH MERCURY.

Mercury alone.

Pony no. 103. 340 lbs., 1st paroxysm. 3rd day of disease. Received 50 c.c. $HgCl_2$ (1 in 1000) subcutaneously. Trypanosomes disappeared after the first dose but reappeared the following day and remained in the circulation for three days. The treatment was continued, the pony received 50 c.c. $HgCl_2$ (1 in 1000) for four consecutive days, followed by 20 c.c. intravenously for 12 days.

Trypanosomes reappeared 8 days after the treatment ceased. They were absent during the second period of treatment.

Pony no. 104. 560 lbs. A spontaneous case received for treatment in an advanced stage of the disease. Received three injections daily of 50 c.c. $HgCl_2$ (1 in 1000) followed by five injections daily of 35 c.c.

Trypanosomes reappeared in the circulation 4 days after treatment ceased. They were absent during the period of treatment.

The subcutaneous injections were not well absorbed and caused pain and swelling at the seat of injection.

Calomel in full and continuous doses had no effect on the course of the disease. It was tested in several cases.

The combination of atoxyl and mercury was also tested, but the results were inferior to those obtained by the use of atoxyl alone.

Mercury and Atoxyl.

Pony no. 105. 600 lbs. Spontaneous case received for treatment. Was in an advanced stage of the disease. Received on alternate days 50 c.c. of 8 % atoxyl and 20 c.c. $HgCl_2$ (1 in 1000) both intravenously. Trypanosomes disappeared after the first dose. The treatment was continued for fifteen days but had little effect on the course of the disease.

Trypanosomes reappeared in the circulation 5 days after the treatment ceased.

Pony no. 106. 300 lbs., 4th paroxysm. 24th day of disease. Had been treated by another method which failed. The pony was treated for four months with atoxyl and mercury administered intravenously. A total amount of 150 c.c. of 4 % atoxyl and 195 c.c. of $HgCl_2$ (1 in 1000) was given.

The treatment had little effect.

TREATMENT WITH ANTIMONY SALTS.

Potassium antimonyl tartrate.

Pony no. 107. Body weight 600 lbs., 2nd paroxysm. 100 c.c. of 1 % solution. Trypanosomes were absent from the blood for seven days.

Pony no. 108. Body weight 600 lbs., 3rd paroxysm. 100 c.c. of 1 % solution. Trypanosomes were absent from the circulation for nine days.

Pony no. 109. Body weight 630 lbs., 4th paroxysm. 25th day of disease. This pony was treated for six weeks receiving at intervals intravenous injections of potassium antimonyl tartrate 1 % solution. The total amount administered was 9·2 gms. During the treatment the pony improved in condition and the circulation remained free from trypanosomes. The trypanosomes reappeared in the blood three weeks after the treatment ceased.

Potassium antimonyl tartrate administered in bolus had no influence on the course of the disease.

Tartar emetic and mercury.

Pony no. 110. Body weight 260 lbs., 3rd paroxysm. 21st day of disease.

1st	day	50 c.c.	of 1 $^0/_0$ tartar emetic	intravenously.
2nd	,,	20	(1 in 1000) $HgCl_2$	,,
3rd	,,	,,	,,	,,
4th	,,	,,	,,	,,
5th	,,	50 c.c.	of 1 $^0/_0$ tartar emetic	,,
6th	,,	20	(1 in 1000) $HgCl_2$	,,
7th	,,	,,	,,	,,
8th	,,	,,	,,	,,
9th	,,	,,	,,	,,
10th	,,	,,	,,	,,
11th	,,	,,	,,	,,

Trypanosomes were absent from the blood from 2nd to 11th day. Reappeared on 12th day.

Pony no. 111. Body weight 250 lbs., 3rd paroxysm. 15th day of disease

1st	day	50 c.c.	of 1 $^0/_0$ tartar emetic	intravenously.
2nd	,,	20	(1 in 1000) $HgCl_2$	,,
3rd	,,	,,	,,	,,
4th	,,	,,	,,	,,
5th	,,	,,	,,	,,
6th	,,	,,	,,	,,
7th	,,	50 c.c.	of 1 $^0/_0$ tartar emetic	,,
8th	,,	,,	,,	,,
9th	,,	100 c.c.	,,	,,

Trypanosomes were absent from the blood 2nd to 9th day. Reappeared 17 days after the treatment ceased.

Tartar emetic and atoxyl.

Pony no. 112. Body weight 600 lbs., 1st paroxysm. 5th day of disease.

1st	day	50 c.c. of 4 $^0/_0$ atoxyl.
3rd	,,	1 gm. of tartar emetic in bolus.
5th	,,	50 c.c. of 4 $^0/_0$ atoxyl.
7th	,,	1 gm. of tartar emetic in bolus.
9th	,,	50 c.c. of 4 $^0/_0$ atoxyl.
11th	,,	1 drachm of tartar emetic in bolus.

Trypanosomes were absent from 2nd to 11th day. Reappeared on 12th day.

Pony no. 113. Body weight 300 lbs., 2nd paroxysm. 13th day of disease. This pony was treated with tartar emetic and atoxyl for a period of six weeks, receiving:

200	c.c. of	4 $^0/_0$	atoxyl	intravenously.
620	,,	1	tartar emetic	intravenously.
525	,,	2	,,	,,

The animal improved in condition and the trypanosomes were absent from the blood during the period of treatment.

A month after the treatment was discontinued the parasites reappeared in the circulation.

Tartar emetic and arsenious acid solution.

Arsenious acid (Loeffler and Ruehs' solution) was given in drench, and tartar emetic 1 $^0/_0$ solution was given intravenously.

Pony no. 114. Body weight 300 lbs., 1st paroxysm. 3rd day of disease.

1st day	25 c.c.	tartar emetic.	11th day	300 c.c.	ars. ac. sol.
3rd ,,	300 ,,	ars. ac. sol.	13th ,,	25 ,,	tartar emetic.
5th ,,	25 ,,	tartar emetic.	15th ,,	300 ,,	ars. ac. sol.
7th ,,	300 ,,	ars. ac. sol.	17th ,,	25 ,,	tartar emetic.
9th ,,	25 ,,	tartar emetic.	19th ,,	300 ,,	ars. ac. sol.

The trypanosomes were absent during the period of treatment, but reappeared 6 days after the last dose.

Tartar emetic, atoxyl and arsenious acid.

Pony no. C 32. Body weight 660 lbs., 2nd paroxysm. 24th day of disease. This animal had received other treatment which failed. Atoxyl was given subcutaneously, tartar emetic intravenously and orpiment in bolus.

1st day	150 c.c. of 4 $^0/_0$ atoxyl.	12th day	100 c.c. of 4 $^0/_0$ atoxyl.
3rd ,,	2 gms. of ars. ac.	13th ,,	50 ,, 1 ,, tartar emetic.
5th ,,	125 c.c. of 4 $^0/_0$ atoxyl.	14th ,,	2·0 gms. of ars. ac.
6th ,,	50 ,, 1 ,, tartar emetic.	18th ,,	100 c.c. of 4 $^0/_0$ atoxyl.
7th ,,	2·0 gms. of ars. ac.	19th ,,	50 ,, 1 ,, tartar emetic.
8th ,,	100 c.c. of 4 $^0/_0$ atoxyl.	20th ,,	2·0 gms. of ars. ac.
9th ,,	50 ,, 1 ,, tartar emetic.	21st ,,	100 c.c. of 4 $^0/_0$ atoxyl.
10th ,,	2·0 gms. of ars. ac.		

No reappearance of trypanosomes in the blood after the second day. Kept under observation for 12 months. The curative effect was probable due to the atoxyl and arsenious acid.

Tartar emetic, arsenious acid solution and arsenious acid.

Arsenious acid (1 in 1000) solution was given in drench, arsenious acid in bolus and tartar emetic 1 $^0/_0$ solution intravenously.

Pony no. 115. Body weight 280 lbs., 1st paroxysm. 7th day of disease.

1st day	250 c.c.	ars. ac. sol.	8th day	25 c.c.	tartar emetic.
2nd ,,	25 ,,	tartar emetic.	9th ,,	300 ,,	ars. ac. sol.
3rd ,,	1·0 gm.	ars. ac.	10th ,,	25 ,,	tartar emetic.
7th ,,	300 c.c.	ars. ac. sol.	11th ,,	1·0 gm.	ars. ac.

Trypanosomes were absent from the blood during period of treatment. They reappeared 15 days after the last dose.

Sodium antimonyl tartrate.

Pony no. 116. Body weight 250 lbs., 1st paroxysm. 50 c.c. of 1 % solution intravenously. Trypanosomes were absent from the blood on following day. They reappeared after eight days.

Pony no. 117. Body weight 260 lbs., 1st paroxysm. 50 c.c. of 1 % solution intravenously. Was off feed and suffered from colic same and following day. Trypanosomes were absent from the blood for five days.

Mule no. 118. Body weight 500 lbs., 1st paroxysm. 100 c.c. of 1 % solution intravenously. Trypanosomes were absent for six days.

Pony no. 119. Body weight 240 lbs., 1st paroxysm. 150 c.c. of 0·5 % solution subcutaneously. Animal was off feed for two days. Swelling and heat at the seat of inoculation for several days. Trypanosomes were absent for five days.

Pony no. 120. Body weight 260 lbs., 1st paroxysm. 100 c.c. of 1 % solution subcutaneously. The dose was followed by colic diarrhoea, and swelling at the seat of inoculation. Thickening of the skin. Symptoms persisted for several days. Trypanosomes were absent for six days.

Aniline antimonyl tartrate.

Pony no. 121. Body weight 250 lbs., 1st paroxysm. 100 c.c. of 1 % solution subcutaneously. Swelling and heat at seat of inoculation. Trypanosomes were absent from the circulation for eight days.

Pony no. 122. Body weight 270 lbs., 1st paroxysm. 100 c.c. of 1 % solution subcutaneously. Off feed, dull, swelling at seat of inoculation. After six days the skin sloughed. Trypanosomes were absent for nine days.

Lithium antimonyl tartrate.

Pony no. 123. Body weight 400 lbs., 1st paroxysm. 50 c.c. of 1 % solution intravenously. Trypanosomes were absent for four days.

Pony no. 124. Body weight 260 lbs., 1st paroxysm. 100 c.c. of 1 % solution intravenously. Fed slowly and had colic on the following day. Trypanosomes were absent for nine days.

Pony no. 125. Body weight 400 lbs., 2nd paroxysm. 100 c.c. of 1 % solution subcutaneously. Off feed, swelling and pain at the seat of inoculation. Trypanosomes were absent for four days.

Pony no. 126. Body weight 200 lbs., 1st paroxysm. 100 c.c. of 1 % solution subcutaneously. Off feed, swelling and pain at the seat of inoculation followed by necrosis of the skin. Trypanosomes were absent for nine days.

List of surra animals which have shown no return of the disease after treatment.

The date of disease at which treatment was commenced is reckoned from the day on which trypanosomes appeared in the circulation.

The period of observation is dated from the completion of the treatment.

All the animals mentioned in this list are still alive and in good condition and health, and no trypanosomes have been observed in their blood for the periods mentioned.

Daily examinations of the blood were made.

No. of animal	Day of disease	Paroxysm	Drugs used	Period under observation
Horse C 1	17th	3rd	ars. ac. alone	6 months
,, C 2	19th	3rd	,,	6 ,,
Pony C 3	25th	4th	,,	3½ ,,
,, C 4	20th	3rd	,,	3 ,,
,, C 5	6th	1st	,,	8 ,,
,, C 6	19th	3rd	,,	10½ ,,
,, C 7	6th	1st	,,	5¼ ,,
,, C 8	14th	1st	,,	3¼ ,,

(1*st method of dosage.*)

No. of animal	Day of disease	Paroxysm	Drugs used	Period under observation
Mule C 9	13th	2nd	Atoxyl & ars. ac.	7½ months
,, C 10	12th	2nd	,,	7½ ,,
Pony C 11	5th	...	,,	4 ,,
,, C 12	4th	1st	,,	10 ,,
,, C 13	5th	1st	,,	10 ,,
,, C 14	1st	1st	,,	14 ,,
,, C 15	5th	1st	,,	12 ,,
,, C 16	1st	1st	,,	12 ,,
Horse C 17	Spontaneous case in advanced stage	—	,,	12 ,,

(2*nd method of dosage.*)

Pony C 18	4th	1st	Atoxyl & ars. ac.	7½ months
,, C 19	18th	3rd	,,	9 ,,
,, C 20	20th	4th	,,	9½ ,,
,, C 21	5th	1st	,,	10 ,,
,, C 22	8th	2nd	,,	8 ,,
,, C 23	13th	2nd	,,	9 ,,
,, C 24	13th	2nd	,,	9 ,,
,, C 25	13th	2nd	,,	9 ,,
,, C 26	13th	2nd	,,	9 ,,
,, C 27	13th	2nd	,,	9 ,,
,, C 28	13th	2nd	,,	8½ ,,
,, C 29	2nd	1st	,,	11 ,,
,, C 30	21st	3rd	arsacetin	8½ ,,
,, C 31	22nd	3rd	soamin	8½ ,,
,, C 32	24th	2nd	atoxyl, tartar emetic and ars. ac.	12 ,,

A summary showing the comparative value of the preparations experimented with in clearing the peripheral circulation from trypanosomes.

A single dose of each preparation was given when the trypanosomes were numerous in the blood and on the 1st or 2nd day of paroxysm.

Except where otherwise stated, the parasites were absent from the circulation the day following the administration of the dose.

The effect of the drug was estimated by the period during which the blood remained free from trypanosomes.

Atoxyl.

No. of animal	Weight in lbs.	Amount of drug	Method administered	Periods
Pony 127	250	30 c.c. of 4 %	subcutaneous	9 days
,, 128	280	30 ,,	,,	7 ,,
,, 129	350	200 ,, (arsacetin)	,,	Cured 8½ months
,, 130	300	100 c.c. of 2 %	,,	4 days
,, ,,	300	150 ,,	,,	6 ,,
,, ,,	300	200 ,,	,,	7 ,,
Mule 131	450	100 ,,	,,	6 ,,
,, ,,	450	200 ,,	,,	7 ,,
,, 132	500	100 ,,	,,	3 ,,
,, ,,	500	150 ,,	,,	5 ,,
,, ,,	500	200 ,,	,,	8 ,,
,, 133	600	40 c.c. of 4 %	intravenous	6 ,,
Pony 134	660	50 ,, 12	,,	5 ,,
,, 135	300	4·8 gms.	by mouth	No effect
,, 136	229	3·0 ,,	,,	,,
,, 137	300	6·0 ,,	,,	Died in 17 hrs.

Soamin.

Mule 138	550	100 c.c. of 10 %	subcutaneous	12 days
,, 139	530	100 ,, 4	,,	6 ,,
Pony 140	350	60 ,,	,,	No effect
,, 141	407	60 ,,	,,	,,
,, C 31	250	100 ,, 10 %	,,	Cured 8½ months
,, 143	550	100 ,, 4	intravenous	No effect
,, 144	250	3·0 gms.	by mouth	,,
,, ,,	250	5·0 ,,	,,	,,
,, ,,	250	6·0 ,,	,,	,,
,, 145	260	10·0 ,,	,,	13 days

Arsenious acid.

No. of animal	Weight in lbs.	Amount of drug	Method administered	Periods
Pony 146	350	1·0 gm.	by mouth	14 days
,, 147	300	1·0 ,,	,,	34 ,,
Mule 148	500	1·0 ,,	,,	
,, 149	480	3·0 gms.	,,	50 ,, (probably cured)
Horse 150	900	1 gm. (1 in 250) sol.	intrav.	16 days
Mule 151	550	0·5 gm. (1 in 1000) sol.	,,	12 ,,
Pony 152	300	1·0 ,,	by mouth	10 ,,
,, 153	350	1·0 ,,	,,	23 ,,
,, C 7	300	1·0 ,,	,,	Cured 5¼ months
,, 155	360	1·0 ,,	,,	14 days
,, 156	300	1·0 ,,	,,	21 ,,
Mule 157	500	2·0 gms.	,,	10 ,,
Pony 158	281	1·0 gm.	,,	12 ,,

Arsenious acid solution (1 *in* 1000).

Pony 159	225	800 c.c.	by mouth	10 days
,, 160	215	800 ,,	,,	14 ,,
Mule 161	500	1200 ,,	,,	5 ,,

Orpiment.

Pony 162	250	6·0 gms.	by mouth	No effect
,, 163	230	7·0 ,,	,,	10 days
,, 164	416	10·0 ,,	,,	8 ,,
,, 165	523	10·0 ,,	,,	7 ,,
,, 166	307	8·0 ,,	,,	5 ,,

Antimony salts. Tartar emetic.

Pony 167	248	100 c.c. of 1 %	intrav.	6 days

Sodium antimonyl tartrate.

Pony 168	400	100 c.c. of 1 %	intrav.	5 days
,, 169	300	100 ,,	subcut.	5 ,,
,, 170	250	50 ,, 0·5 %	,,	4 ,,

Lithium antimonyl tartrate.

Pony 171	260	100 c.c. of 1 %	intrav.	8 days
,, 172	230	100 ,,	,,	4 ,,
,, 173	230	50 ,,	,,	4 ,,

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ON HAEMATOZOA OCCURRING IN WILD ANIMALS IN AFRICA.

1. *PIROPLASMA ROSSI* N.SP. AND *HAEMOGREGARINA CANIS ADUSTI* N.SP. FOUND IN THE JACKAL.

2. *SPIROCHAETA BOVIS CAFFRIS* N.SP. FOUND IN THE BUFFALO.

BY GEORGE H. F. NUTTALL, F.R.S.

Plates X and XI.

OWING to the courtesy of my friend, Mr W. F. Cooper, B.A., who prepared blood-films and organ smears from various wild animals shot by him in British East Africa in August, 1909, I am in a position to record three new parasites from the Jackal and Buffalo. Mr Cooper prepared the films as soon as possible after the animals were shot, using either absolute alcohol or heat for fixation. On arrival in Cambridge, the films were stained in the usual manner by Giemsa.

The Jackal (*Canis adustus*) was shot to the S.E. and near to Fort Hill, about four miles on the road to Punda Milia. The Buffalo (*Bos caffer typicus*) was shot about five miles to the north of Punda Milia. I shall first describe the two protozoal parasites encountered in the Jackal.

The Parasites of the Jackal.

The parasites were encountered in the course of a very careful and systematic examination of eight coverglass smear preparations made from the spleen (4) and liver (4) of the jackal. Altogether, the smeared coverglasses represented an area of 12 square centimetres and it took many days to thoroughly examine them with the aid of a mechanical stage. My laboratory assistant, Mr B. G. Clarke, aided me considerably in the search for parasites. In all, we discovered 98 parasites, of which eight were haemogregarines.

Piroplasma rossi n.sp.

Plate X.

It is with hesitation that I place this parasite in the genus *Piroplasma*, and I only do so provisionally for reasons about to be described. On the other hand, it appears safe to refer the protozoon to the Family *Piroplasmidae* of França. Were it not for the fact that I have recently discovered somewhat similar parasites in the internal organs of a dog 235 days after it had been inoculated with *Piroplasma canis*, the dog having recovered in consequence of trypanblue treatment (see *Parasitology*, Vol. II. p. 180), I should unhesitatingly have referred the parasite of the jackal to a new genus. Only two parasites were encountered in the dog, and it will be necessary to examine other animals before any conclusions can be reached regarding the resemblance existing between the parasites. The parasites of the jackal differ very markedly from *P. canis* as observed in the peripheral blood during the normal course of piroplasmosis. Thus, in the jackal parasite there is an absence of the typical pairs of pyriforms and the characteristic dividing ("budding") forms are lacking; the nucleus is, moreover, very much larger and differs from that of *P. canis*, as elsewhere described by Dr Graham-Smith and myself. When we come to examine smears from the organs of dogs, long after the animals have "recovered," with the same care that we have devoted to the examination of the smears from the jackal, we shall be in a better position to express an opinion on the subject. It is conceivable that the parasites of the jackal represent a special form of *Piroplasma* occurring in animals which suffer from a chronic infection or which have recovered from acute piroplasmosis. To my knowledge parasites have not hitherto been described from animals after "recovery" from piroplasmosis. We know that they are present in peripheral blood because the blood of "salted" animals is infective for months or years although failure has attended the efforts made to detect the parasites by microscopic examinations. Assuming, on the other hand, that the parasite of the jackal is a *Piroplasma* of an hitherto undescribed type and peculiar to recovered animals, it may well be referred to a new species in view of the host in which it is found, for, as we have recorded elsewhere, the African *P. canis* appears to be peculiarly specific since infection experiments made with it upon jackals and foxes (*Parasitology*, Vol. II. p. 211) have given negative results. Should future investigation prove that the parasite of the jackal differs from the types of *P. canis* encountered in "salted" dogs, I would propose to refer it to a new genus *Rossiella*[1] (*Rossia* is preoccupied) in view of its specific name.

The following description is based upon the examination of 90 parasites encountered after a complete search had been made of the stained preparations at my disposal. The spleen contained 84 parasites, the liver smears only six parasites.

P. rossi occurs as a large intracorpuscular parasite of rounded, ovoid, sub-pyriform or irregular shape, possessing a single large nucleus, and occurring singly, in pairs or in fours, within the infected corpuscles. The parasite produces no pigment and appears to multiply by simple division into two parasites, which may in turn subdivide. Simple division of the nucleus precedes the separation into daughter cells. Judging from the stained preparations there is evidence that the parasite is capable of amoeboid movement at various stages of its development. The parasite causes no apparent changes in the corpuscles which it inhabits.

Measurements. The smallest uninucleate parasites measured $1\cdot5\,\mu$, the largest $3\cdot3 \times 5\cdot3\,\mu$. Since the large parasites subdivide to form two or four smaller parasites intermediate sizes are encountered with nuclei of corresponding size. Both the infected and uninfected red blood corpuscles varied in size, from 6 to $9\,\mu$ in diameter.

General structure. The general form of the parasite varies considerably. It may be spherical and sharply defined (Pl. X, Figs. 1, 5, 16) or ovoid (Figs. 4, 10, 25) to sub-pyriform (Figs. 7, 13, 14), or it may be irregular in outline owing to the protrusion of amoeboid processes which are lobose (Figs. 3, 15) or more or less pointed (Figs. 8, 12, 19, 26). The *protoplasm* takes a clear blue colour, when stained by Giemsa, with a tendency for the staining to be more intense at the periphery, as in *Piroplasma*, but there is less evidence of vacuolar structure; in fact the protoplasm may at times assume a uniformly blue tint. In all the parasites a clear unstained halo surrounds the chromatin mass. At times clear vacuolar areas are seen in the blue staining protoplasm (Figs. 1, 3, 15), at other times fairly definite achromatic lines traverse the parasite (Figs. 16, 17). The *nucleus* is striking because of its relatively large size. It is situated centrally or peripherally; in the latter case it is in most instances bounded on the outside by a band of blue-staining protoplasm. The nucleus is usually of rounded or oval form with a sharply defined contour. In some nuclei, fine chromatin particles appeared to be aggregated at the periphery or to be scattered through the pale pink-staining substance of the chromatic nucleus. When nuclear division occurs (see below under Mode of Multiplication) the chromatin appears to be more diffused and the nucleus is of irregular form. In one case the extrusion of what may have been a dividing nucleus was observed (Fig. 15). In this case the chromatin is peripherally distributed as though surrounding a vacuole, but the appearance may be unnatural, especially as the blue-staining parasite itself appears "blistered," as is occasionally seen in *Piroplasma* in imperfectly stained films.

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[1] In honour of Professor Ronald Ross, C.B., F.R.S.

Mode of Multiplication. The manner in which the parasite undergoes division within the corpuscle appears to be as follows: when a single parasite (Pl. X, Figs. 1—4) has attained a certain size, its nucleus divides (Figs. 5—6) and this is followed by division of the parasite into two (Figs. 7—12). The newly-formed pair of parasites, if sufficiently mature, may repeat the process (Figs. 15—19), thus giving rise to four parasites within a corpuscle (Figs. 20—22). Nuclear division is simple and clearly precedes the division of the protoplasm both in single parasites (Figs. 5—6) and in pairs of intracorpuscular parasites (Figs. 15—19). In parasites about to divide the chromatin of the nucleus appears to be diffuse (Fig. 4) or drawn out in an irregular thread-like manner (Figs. 13, 14) as if the nucleus were in a state of flux. The chromatin becomes aggregated at opposite ends of the parasite and the connecting strand ruptures (Fig. 15), after which the newly-formed nuclei become rounded (Figs. 15—19).

In view of the small number of parasitized corpuscles which could be discovered in the film it is impossible to say if more than four parasites may be formed in the corpuscle. Judging, however, from the absence of smaller forms than those figures (Figs. 18, 23, 29) it is reasonable to assume that not more than four parasites are formed within a corpuscle.

Enumeration of the Types of Parasites encountered.

Intracorpuscular parasites. The complete examination of the smears from the jackal only revealed 90 parasites. Sketches and notes were made of all the parasites encountered. In ordering the data thus collected it was found that the majority of the infected corpuscles (81 °/₀) contained one, two or four discrete parasites, whereas the remaining corpuscles contained parasites in various stages of development, as shown (bracketed "D") in the subjoined table:

	No. of r.b.c.	which contained parasites as follows	As figured in Plate X
81 %	33	2 parasites each with a single nucleus.	Figs. 7—12.
	29	1 parasite with a single nucleus.	Figs. 1—4, 23, 24.
	11	4 parasites each with a single nucleus.	Figs. 20—22.
	6	1 parasite with two nuclei.	Figs. 5—6.
9 % (D)	3	2 parasites each with two nuclei.	Figs. 15—17.
	3	2 parasites each with nuclei showing processes.	Figs. 13—14.
	3	3 parasites: two containing single nuclei and one containing two nuclei.	Figs. 18—19.
	2	1 parasite with nucleus showing processes.	As in Fig. 14.

Free parasites. With but one exception all of the parasites which were encountered outside of corpuscles possessed a single nucleus. The one parasite which contained two nuclei (Pl. X, Fig. 28) may have issued prematurely from the corpuscle owing possibly to mechanical injury to the latter in preparing the blood-film. Of the uninucleate free parasites five occurred singly, eight in couples, and four in one group, suggesting in the latter instances that the parasites had just escaped from corpuscles. From the appearance of the films, many corpuscles were injured when the smear was prepared, consequently no great stress can be laid upon the number of free forms encountered. In the absence of observations upon the living parasite, it is impossible to know in what manner the parasites enter the corpuscles they invade.

Haemogregarina canis adusti n.sp.

Plate XI, Figs. 1—5.

This parasite, of which only eight specimens were discovered in smears from the jackal's spleen, appears to be the first haemogregarine recorded as occurring in an African carnivore. The parasites measured 10 × 4·3 μ to 13 × 6·3 μ in size. Three of the eight parasites were contained in mononuclear leucocytes whose nuclei appeared compressed or distorted from contact with the parasite (Plate XI, Figs. 4, 5). Some of the parasites showed a distinct cytocyst. Otherwise there is nothing special to note. I herewith append a list of the haemogregarines occurring in mammals; all of them have been discovered of recent years. Whereas some of them occur in red blood corpuscles, others occur in mononuclear leucocytes (whence frequently termed *Leucocytozoa*) during the stage of their development when they are found in the peripheral blood:

Haemogregarina occurring in Leucocytes.

Species and Synonymy	Host	Country	Size of parasite
H. canis (James), 1905 =*L. bentleyi* Adie, 1906	*Canis familiaris*	Assam (Bentley, 1904). Perak, Fed. Malay States (Gerrard, 1906), India	8—10 × 4—5 μ (Bentley, 1905) 11—12 × 4·2—5 μ (Gerrard)
H. canis adusti Nuttall, 1910	*Canis adustus* (Jackal)	British East Africa	10—13 × 4·3—6·3 μ
H. felis domesticae (Patton), 1908	*Felis domesticus*	Madras, India	Not given
H. muris Balfour, 1905–6	*Mus decumanus*	Sudan (Balfour). Perth, W. Australia (Cleland, 1906)	9—10·5 × 4·5 μ
=(?) *L. ratti* Adie, 1906	*Mus rattus*	India	11·7 × 5·6 μ
=(?) *H. perniciosa* (Miller), 1908 =*Hepatozoon perniciosum*	*Mus decumanus* (white rat)	United States of N. America	12 × 6 μ
H. musculi (Porter), 1908	*Mus musculus*	London	10·9 × 5·1 μ
H. funambuli (Patton), 1906	*Funambulus palmarum* (palm squirrel)	India	10 × 5 μ
H. leporis (Patton), 1908	*Lepus nigricollis* (hare)	Madras, India	Not given

Haemogregarina occurring in Red Blood Corpuscles.

Species	Host	Country
H. balfouri Laveran, 1905 =*H. jaculi* Balfour, 1906	*Jaculus gordoni* Jerboa	Sudan
H. gerbilli Christophers, 1905	*Gerbillus indicus* Indian field rat	India
H. dasyuri Welsh, Dalyell & Burfitt, 1908	*Dasyurus viverrinus* "native cat"	Sidney, Australia
H. petauri Welsh & Barling, 1908	*Petaurus sciureus* marsupial flying squirrel	Sidney, Australia

Spirochaeta bovis caffris n.sp.

Plate XI, Figs. 6—9.

The examination of four coverglass preparations made from the blood of the buffalo (p. 108) revealed only four spirochaete-like parasites, all of which are illustrated in the accompanying plate. They differ from any spirochaetes I have hitherto seen in being very broad although they taper uniformly at both ends. Stained by Giemsa, they assumed a violet or violet-blue tint. Whereas the parasite depicted in Fig. 6 shows no internal structure, the others contain very fine chromatin particles. In one parasite (Fig. 9) transverse achromatic bands are observable. Although the parasites vary somewhat in size they are remarkably uniform in shape. I do not know what else to call them than spirochaetes. Their form suggests that in life they may possess an eel-like movement, but this appears unlikely from the fact that the four parasites are all curved similarly. They measured 20 to 25 μ by 1 to 1·5 μ.

It is possible that these parasites belong in the same category as those described and figured in a photomicrograph by Bowhill (1905, p. 7, Pl. XI, Fig. 8) and which he regarded as larval nematodes in the blood of a horse at the Cape. Bowhill's parasites measure approximately 15 μ in length, and, judging from his figure, possess darkly stained granular protoplasm. They appear to have been very numerous in the blood of the horse, which was suffering from biliary fever.

The buffalo parasite also recalls forms described by Edm. and Et. Sergent (1903, p. 1163) and roughly figured by them. The Sergent parasites were discovered in the blood of a native of Algiers who suffered from attacks of perspiration every night but appeared otherwise normal. The parasites occurred in the blood with a definite periodicity, *i.e.* only during the daytime (8 a.m. to 6 p.m.). The man's symptoms resembled those of an "accés larvé de paludisme." This parasite must be rare, since the Sergents failed to find it before in the course of hundreds of routine blood examinations made in hospital patients. The parasites were not detected in fresh blood-films. In stained preparations they measured 36 to 45 μ by 1 to 1·5 μ. As a rule they tapered uniformly at both ends. They appear to be flattened and fairly rigid structures, the curvatures being slight. Stained by azur-eosin they mostly assumed a pale mauve tint. In some well-stained specimens the middle third of the parasite appeared blueish and granular, in others this part (the nucleus?) of the parasite appeared pale, distinctly granular and surrounded by a refringent line. Not more than 25 parasites were encountered in a single blood-film.

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DESCRIPTION OF PLATES X AND XI.

The drawings were executed in water-colours. They were drawn to scale with the aid of a Winkel Camera-Lucida and Zeiss 2 mm. (oil immersion) objective: using ocular No. 18. The size of the parasites was determined by the use of a Zeiss stage micrometer scale projected upon the paper upon which the drawings were made.

PLATE X.

Piroplasma rossi n.sp.

× 3600.

With the exception of the parasite depicted in Fig. 1, which was found in a liver-smear, all of the parasites figured were encountered in smears from the spleen of the Jackal.

Intracorpuscular parasites.

Figs. 1—3. Uninucleate parasites showing vacuoles and sharply defined or (Fig. 2) diffuse contour with indications of amoeboid movement. Nucleus compact.

Fig. 4. Large ovoid parasite with central nucleus of irregular flowing form with the chromatin diffused.

Figs. 5—6. Large rounded parasites containing two nuclei distant from each other. These parasites are about to divide.

Fig. 7. Two parasites possessing a somewhat pyriform shape.

Fig. 8. Two parasites showing amoeboid movement and diffused chromatin.

Figs. 9—10. Pairs of parasites. Those in Fig. 10 are very large and each shows a large mass of chromatin, the appearance suggesting that the parasites might well be about to divide into two.

Figs. 11—14. Pairs of parasites with diffuse or irregular chromatin. In the parasite to the right in Fig. 14 the chromatin is drawn out in a thread and becoming concentrated in masses at the ends of the thread.

Fig. 15. The parasite to the left appears to have just undergone simple nuclear division, the connecting thread between the flowing chromatin mass having ruptured. In the parasite to the right the nuclei have already become rounded and lie at opposite poles of the parasite. Both parasites show evidence of amoeboid motion.

Figs. 16—17. Pairs of parasites in which the nuclei have subdivided preparatory to the formation of four intracorpuscular parasites. Achromatic lines occur in the blue-staining protoplasm.

Figs. 18—19. Corpuscles containing three parasites, one of which contains two nuclei at opposite ends of the parasite; it represents a dividing form. Each corpuscle contains a pair of parasites which have just undergone division.

Figs. 20—22. Corpuscles containing four parasites, some of which are amoeboid.

Figs. 23—24. Parasites seen in almost dehaemoglobinized corpuscles.

Fig. 25. A parasite presenting a sharply defined bubble-like appearance with the blue-staining protoplasm condensed peripherally and similarly altered (dividing ?) nucleus to one side.

Free Parasites.

Figs. 26—27. Single free parasites of amoeboid form with rounded, compact nuclei and deeply blue-staining protoplasm.

Fig. 28. Single free parasite with two nuclei, the chromatin being diffusely distributed. The parasite was presumably expelled prematurely from the containing corpuscle.

Figs. 29—30. A pair of free parasites and a group of four free parasites which have just escaped from corpuscles. Some of the parasites appear amoeboid.

PLATE XI.

Haemogregarina canis adusti n.sp.

× 3600

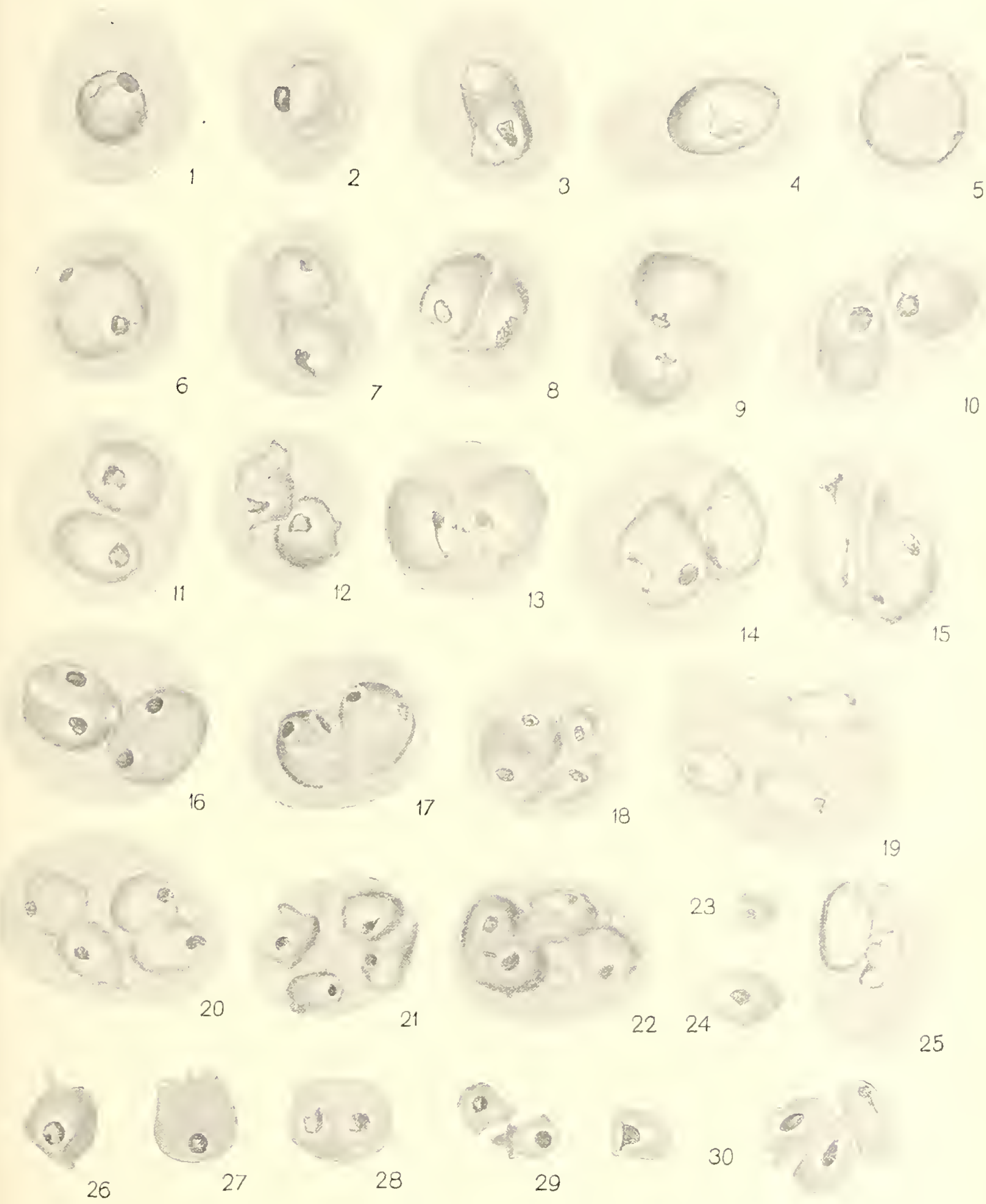
Figs. 1—3. Free parasites. Fig. 3 shows the enveloping membrane or cytocyst and diffuse chromatin; in Fig. 2 there are scattered chromatin points in the protoplasm.

Figs. 4—5. Parasites contained in mononuclear leucocytes whose nuclei are compressed by the body of the parasite. In Fig. 4 the nucleus of the parasite appears reticular.

Spirochaeta bovis caffris n.sp.

× 3600

Figs. 6—9. Spirochaetes showing variation in size but uniformity in shape. Whereas in Fig. 6 the parasite shows no internal structure, in Figs. 7 and 8 the chromatin occurs as fine dust throughout the faintly violet-blue staining protoplasm. In Fig. 9 there are achromatic transverse bands visible.



Piroplasma rossi n. sp.

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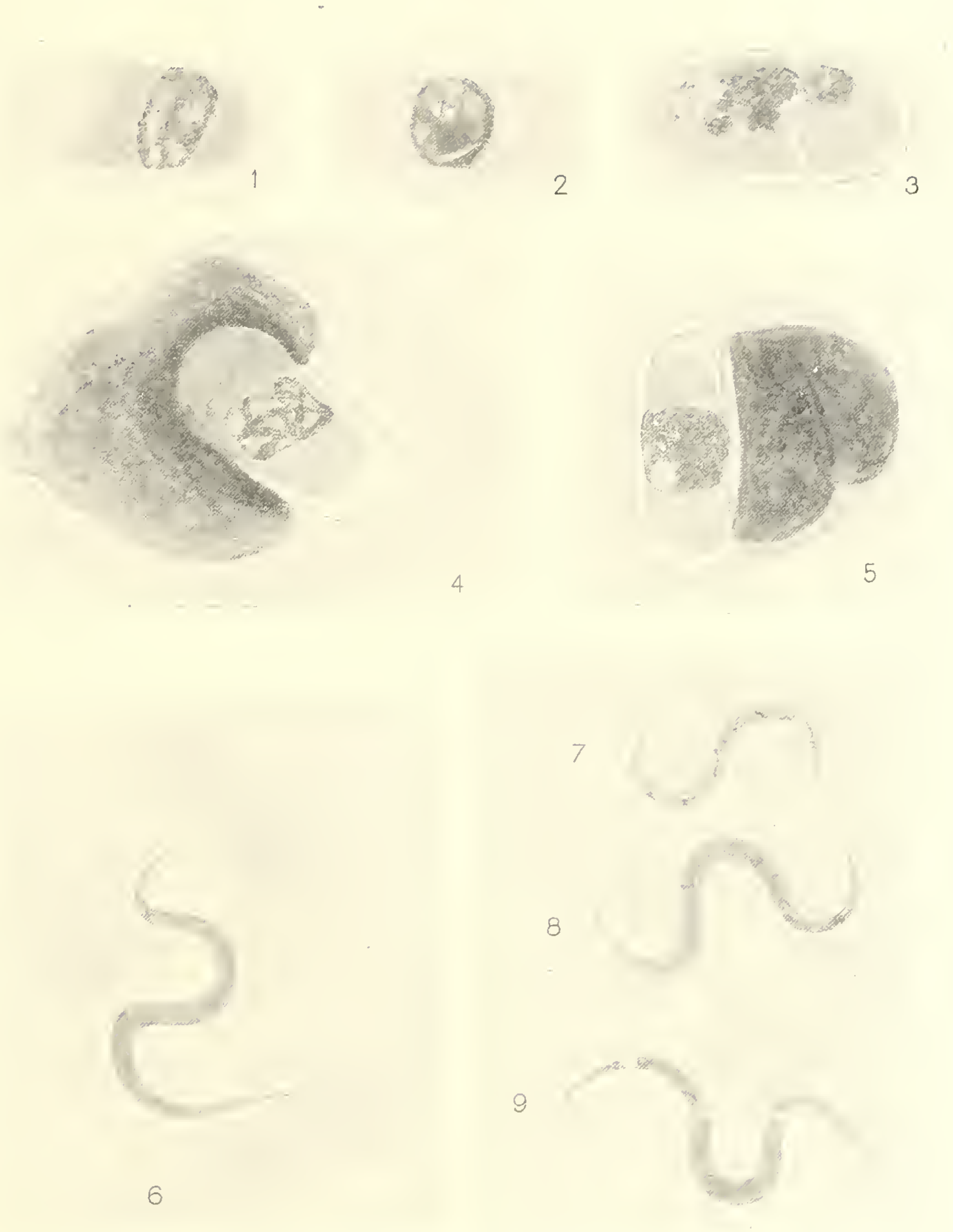

Haemogregarina canis adusti n. sp.
and
Spirochaeta bovis caffris n. sp.

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